	L #	Hits	Search Text	DBs	Time Stamp
1	L1	1348	methionine same cysteine same muta$10	USPAT; US-PGPUB	2003/07/18 09:14
2	L2	92	1 same stab$8	USPAT; US-PGPUB	2003/07/18 09:21
3	L3	39861	oxidat$ same stab$8	USPAT; US-PGPUB	2003/07/18 09:22
4	L4	102	1 and 3	USPAT; US-PGPUB	2003/07/18 09:21
5	L5	17453	oxidat$ near4 stab$8	USPAT; US-PGPUB	2003/07/18 09:22
6	L6	57	1 and 5	USPAT; US-PGPUB	2003/07/18 09:22
7	L7	2404	sulfur adj free	USPAT; US-PGPUB	2003/07/18 10:08
8	L8	1	7 near2 (protein$1 or enzyme$1)	USPAT; US-PGPUB	2003/07/18 10:08

PGPUB-DOCUMENT-NUMBER: 20030086924

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030086924 A1

TITLE: Treatment with anti-ErbB2 antibodies

PUBLICATION-DATE: May 8, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sliwkowski, Mark X.	San Carlos	CA	US	

APPL-NO: 10/ 268501

DATE FILED: October 10, 2002

RELATED-US-APPL-DATA:

child 10268501 A1 20021010

parent continuation-in-part-of 09602812 20000623 US PENDING

non-provisional-of-provisional 60141316 19990625 US

US-CL-CURRENT: 424/143.1, 424/155.1

ABSTRACT:

The present application describes methods for treating cancer with anti-ErbB2 antibodies, such as anti-ErbB2 antibodies that block ligand activation of an ErbB receptor.

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. Ser. No. 09/602,812 filed Jun. 23, 2000, which is a non-provisional application filed under 37 CFR 1.53(b)(1), claiming priority under 35 USC 119(e) to provisional application No. 60/141,316 filed Jun. 25, 1999, the contents of both applications are incorporated herein by reference.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (2):

[0030] FIGS. 1A and 1B depict epitope mapping of residues 22-645 within the extracellular domain (ECD) of ErbB2 (amino acid sequence, including signal sequence, shown in FIG. 1A; SEQ ID NO:13) as determined by truncation **mutant** analysis and site-directed **mutagenesis** (Nakamura et al. J. of Virology

67(10):6179-6191 (1993); and Renz et al. J. Cell Biol. 125(6):1395-1406 (1994)). The various ErbB2-ECD truncations or point **mutations** were prepared from cDNA using polymerase chain reaction technology. The ErbB2 **mutants** were expressed as gD fusion proteins in a mammalian expression plasmid. This expression plasmid uses the cytomegalovirus promoter/enhancer with SV40 termination and polyadenylation signals located downstream of the inserted cDNA. Plasmid DNA was transfected into 293 cells. One day following transfection, the cells were metabolically labeled overnight in **methionine and cysteine**-free, low glucose DMEM containing 1% dialyzed fetal bovine serum and 25 .mu.Ci each of .sup.35S **methionine** and .sup.35S **cysteine**. Supernatants were harvested and either the anti-ErbB2 monoclonal antibodies or control antibodies were added to the supernatant and incubated 2-4 hours at 4.degree. C. The complexes were precipitated, applied to a 10-20% Tricine SDS gradient gel and electrophoresed at 100 V. The gel was electroblotted onto a membrane and analyzed by autoradiography. As shown in FIG. 1B, the anti-ErbB2 antibodies 7C2, 7F3, 2C4, 7D3, 3E8, 4D5, 2H11 and 3H4 bind various ErbB2 ECD epitopes.

Detail Description Paragraph - DETX (137):

[0178] Any cysteine residue not involved in maintaining the proper conformation of the anti-ErbB2 antibody also may be substituted, generally with serine, to improve the **oxidative stability** of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

PGPUB-DOCUMENT-NUMBER: 20030036116

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030036116 A1

TITLE: Exonuclease-mediated nucleic acid reassembly in directed evolution

PUBLICATION-DATE: February 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Short, Jay M.	Rancho Santa Fe	CA	US	

US-CL-CURRENT: 435/69.1, 530/350 , 536/23.2

ABSTRACT:

This invention provides methods of obtaining novel polynucleotides and encoded polypeptides by the use of non-stochastic methods of directed evolution (DirectEvolution.TM.). A particular advantage of exonuclease-mediated reassembly methods is the ability to reassemble nucleic acid strands that would otherwise be problematic to chimerize. Exonuclease-mediated reassembly methods can be used in combination with other mutagenesis methods provided herein. These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). This invention provides methods of obtaining novel enzymes that have optimized physical &/or biological properties. Through use of the claimed methods, genetic vaccines, enzymes, small molecules, and other desirable molecules can be evolved towards desirable properties. For example, vaccine vectors can be obtained that exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like. Furthermore, this invention provides methods of obtaining a variety of novel biologically active molecules, in the fields of antibiotics, pharmacotherapeutics, and transgenic traits.

PGPUB-DOCUMENT-NUMBER: 20020172661

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020172661 A1

TITLE: HSA- free formulations of interferon-beta

PUBLICATION-DATE: November 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Shirley, Bret A.	Waltham	MA	US	
Babuka, Susan	Oakland	CA	US	
Chen, Bao-Lu	San Ramon	CA	US	
Hora, Maninder	Danville	CA	US	
Choe, Minna	Danville	CA	US	
Tellers, Melanie	Cranford	NJ	US	

APPL-NO: 10/ 035397

DATE FILED: October 25, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60282614 20010409 US

non-provisional-of-provisional 60330404 20011018 US

US-CL-CURRENT: 424/85.6

ABSTRACT:

Stabilized pharmaceutical compositions comprising substantially monomeric interferon-beta (IFN-.beta.) and methods useful in their preparation are provided. The compositions comprise the IFN-.beta. solubilized in a low-ionic-strength formulation that maintains the composition at a pH of about 3.0 to about 5.0. Methods for preparing these compositions, and for increasing solubility of IFN-.beta. in pharmaceutical compositions, are provided.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application Serial No. _____, filed Oct. 18, 2001, entitled "HSA-Free Formulations of Interferon-Beta," and U.S. Provisional Application Serial No. 60/282,614, filed Apr. 9, 2001, each of which is herein incorporated by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX (19):

[0051] The IFN-.beta. variants encompassed herein include muteins of the mature native IFN-.beta. sequence, wherein one or more **cysteine** residues that are not essential to biological activity have been deliberately deleted or replaced with other amino acids to eliminate sites for either intermolecular crosslinking or incorrect intramolecular disulfide bond formation. IFN-.beta. variants of this type include those containing a glycine, valine, alanine, leucine, isoleucine, tyrosine, phenylalanine, histidine, tryptophan, serine, threonine, or **methionine** substituted for the **cysteine** found at amino acid 17 of the mature native amino acid sequence. Serine and threonine are the more preferred replacements because of their chemical analogy to **cysteine**. Serine substitutions are most preferred. In one embodiment, the **cysteine** found at amino acid 17 of the mature native sequence is replaced with serine. **Cysteine** 17 may also be deleted using methods known in the art (see, for example, U.S. Pat. No. 4,588,584, herein incorporated by reference), resulting in a mature IFN-.beta. mutein that is one amino acid shorter than the mature native IFN-.beta.. See also, as examples, U.S. Pat. Nos. 4,530,787; 4,572,798; and 4,588,585. Thus, IFN-.beta. variants with one or more **mutations** that improve, for example, their pharmaceutical utility are also encompassed by the present invention.

Detail Description Paragraph - DETX (40):

[0072] In addition to those agents disclosed above, other stabilizing agents, such as ethylenediaminetetracetic acid (EDTA) or one of its salts such as disodium EDTA, can be added to further enhance the stability of the liquid pharmaceutical compositions. The EDTA acts as a scavenger of metal ions known to catalyze many **oxidation reactions, thus providing an additional stabilizing** agent. Other suitable stabilizing agents include non-ionic surfactants, including polyoxyethylene sorbitol esters such as polysorbate 80 (Tween 80) and polysorbate 20 (Tween 20); polyoxypropylene-polyoxyethylene esters such as Pluronic F68 and Pluronic F127; polyoxyethylene alcohols such as Brij 35; simethicone; polyethylene glycol such as PEG400; lysophosphatidylcholine; and polyoxyethylene-p-t-octylphenol such as Triton X-100. Classic stabilization of pharmaceuticals by surfactants is described, for example, in Levine et al.(1991) J. Parenteral Sci. Technol. 45(3):160-165, herein incorporated by reference.

PGPUB-DOCUMENT-NUMBER: 20020146762

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020146762 A1

TITLE: End selection in directed evolution

PUBLICATION-DATE: October 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Short, Jay M.	Rancho Santa Fe	CA	US	
Frey, Gerhard Johann	San Diego	CA	US	

US-CL-CURRENT: 435/69.1, 435/440 , 435/5 , 435/69.7 , 435/7.6 , 530/350 , 536/23.2

ABSTRACT:

This invention provides methods of obtaining novel polynucleotides and encoded polypeptides by the use of non-stochastic methods of directed evolution (DirectEvolution.TM.). A particular advantage of end-selection-based methods is the ability to recover full-length polynucleotides from a library of progeny molecules generated by mutagenesis methods. These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). This invention provides methods of obtaining novel enzymes that have optimized physical &/or biological properties. Through use of the claimed methods, genetic vaccines, enzymes, small molecules, and other desirable molecules can be evolved towards desirable properties. For example, vaccine vectors, can be obtained that exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like. Furthermore, this invention provides methods of obtaining a variety of novel biologically active molecules, in the fields of antibiotics, pharmacotherapeutics, and transgenic traits.

PGPUB-DOCUMENT-NUMBER: 20020137177

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020137177 A1

TITLE: Modified enzymes and their use for peptide synthesis

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Jones, J. Bryan	Lakefield		CA	

APPL-NO: 10/ 075895

DATE FILED: February 13, 2002

RELATED-US-APPL-DATA:

child 10075895 A1 20020213

parent division-of 09234957 19990121 US GRANTED

parent-patent 6395532 US

non-provisional-of-provisional 60072351 19980123 US

non-provisional-of-provisional 60072265 19980123 US

US-CL-CURRENT: 435/219, 435/252.3 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

The present invention relates to modified enzymes with one or more amino acid residues from an enzyme being replaced by cysteine residues, where at least some of the cysteine residues are modified by replacing thiol hydrogen in the cysteine residue with a thiol side chain to form a modified enzyme, wherein the modified enzyme has high esterase and low amidase activity. Also, a method of producing the modified enzymes is provided. The present invention also relates to a method for using the modified enzymes in peptide synthesis.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 60/072,351, filed Jan. 23, 1998, and U.S. Provisional Patent Application Serial No. 60/072,265, filed Jan. 23, 1998, and which are hereby incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (7):

[0006] U.S. Pat. No. 5,208,158 to Bech et al. ("Bech") describes chemically modified detergent enzymes where one or more methionines have been mutated into cysteines. The cysteines are subsequently modified in order to confer upon the enzyme improved **stability towards oxidative** agents. The claimed chemical modification is the replacement of the thiol hydrogen with C.sub.1-6 alkyl.

Summary of Invention Paragraph - BSTX (8):

[0007] Although Bech has described altering the **oxidative stability** of an enzyme through mutagenesis and chemical modification, it would also be desirable to develop one or more enzymes with altered properties such as activity, nucleophile specificity, substrate specificity, stereoselectivity, thermal stability, pH activity profile, and surface binding properties for use in, for example, detergents or organic synthesis. In particular. enzymes, such as subtilisins, tailored for peptide synthesis would be desirable. Enzymes useful for peptide synthesis have high esterase and low amidase activities. Generally, subtilisins do not meet these requirements and the improvement of the esterase to amidase selectivities of subtilisins would be desirable. However, previous attempts to tailor enzymes for peptide synthesis by lowering amidase activity have generally resulted in dramatic decreases in both esterase and amidase activities. Previous strategies for lowering the amidase activity include the use of water-miscible organic solvents (Barbas et al., J. Am. Chem. Soc., 110:5162-5166 (1988); Wong et al., J. Am. Chem. Soc., 112:945-953 (1990); and Sears et al., Biotechnol. Prog., 12:423-433 (1996)) and site-directed mutagenesis (Abrahamsen et al., Biochemistry, 30:4151-4159 (1991); Bonneau et al., J. Am. Chem. Soc., 113:1026-1030 (1991); and Graycar et al., Ann. N.Y. Acad. Sci., 67:71-79 (1992)). However, while the ratios of esterase-to-amidase activities were improved by these approaches, the absolute esterase activities were lowered concomitantly. Abrahamsen et al., Biochemistry, 30:4151-4159 (1991). Chemical modification techniques (Neet et al., Proc. Nat. Acad. Sci., 56:1606 (1966); Polgar et al., J. Am. Chem. Soc., 88:3153-3154 (1966); Wu et al., J. Am. Chem. Soc., 111:4514-4515 (1989); and West et al., J. Am. Chem. Soc., 112:5313-5320 (1990)), which permit the incorporation of unnatural amino acid moieties, have also been applied to improve esterase to amidase selectivity of subtilisins. For example, chemical conversion of the catalytic triad serine (Ser221) of subtilisin to cysteine (Neet et al., Proc. Nat. Acad. Sci., 56:1606 (1966); Polgar et al., J. Am. Chem. Soc., 88:3153-3154 (1966); and Nakatsuka et al., J. Am. Chem. Soc., 109:3808-3810 (1987)) or to selenocysteine (Wu et al., J. Am. Chem. Soc., 111:4514-4515 (1989)), and methylation of the catalytic triad histidine (His57) of chymotrypsin (West et al., J. Am. Chem. Soc., 112:5313-5320 (1990)), effected substantial improvement in esterase-to-amidase selectivities. Unfortunately however, these modifications were again accompanied by 50-to 1000-fold decreases in absolute esterase activity.

Detail Description Paragraph - DETX (74):

[0077] At the Met222 site, both M222C--SCH.sub.2CH.sub.2NH.sub.3.sup.+ (-h) and M222C exhibited an improved esterase k.sub.cat/K.sub.M of up to 1.5, while

all of M222C--S--CH.sub.3 (-a), M222C--SCH.sub.2CH.sub.2NH.sub- .3.sup.+ (-h), and M222C displayed up to 37-fold reduced amidase activity. The esterase-to-amidase activity of the **cysteine** parent, M222C, with its 4-fold improvement, was itself significantly higher than WT. The M222C **mutant** has a S.sub.1' leaving group site that is less sterically congested than WT. This may enhance the rate of acyl-enzyme hydrolysis, which is often the rate-determining step for ester substrates. M222C--S--CH.sub.3 (-a), which differs from WT only in the replacement of one of the **methionine** side-chain methylenes (CH.sub.2) by sulfur, had the same k.sub.cat as WT, but an increased K.sub.M. At this site, the most improved ME was M222C--SCH.sub.2CH.sub.2NH.sub.3.sup.+ (-h), which exhibited an esterase-to-amidase selectivity of 879, compared to 17 for the WT. This 52-fold improvement in esterase-to-amidase ratio of the series arose largely from a 31 -fold lowered amidase k.sub.cat, but with the WT level of esterase k.sub.cat being retained. This result was consistent with the observation that the M222K **mutant** of subtilisin BPN' caused improved esterase activity and severely decreased amidase activity, thus, generating an enzyme with greatly improved esterase-to-amidase specificity. Graycar et al., Ann. N.Y. Acad. Sci., 672:71-79 (1992), which is hereby incorporated by reference.

PGPUB-DOCUMENT-NUMBER: 20020114782

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020114782 A1

TITLE: Stabilized interferon compositions

PUBLICATION-DATE: August 22, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Wolfe, Sidney N.	Lafayette	CA	US	
Hora, Maninder S.	Danville	CA	US	

APPL-NO: 10/ 010448

DATE FILED: November 7, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60246456 20001107 US

non-provisional-of-provisional 60252224 20001121 US

US-CL-CURRENT: 424/85.6

ABSTRACT:

Stabilized pharmaceutical formulations comprising IFN-.beta. and highly purified mannitol are provided. The highly purified mannitol stabilizes the compositions by reducing the formation of IFN-.beta. adducts in comparison with IFN-.beta. formulated with mannitol that has not been highly purified. Methods for increasing the stability of IFN-.beta. or a variant thereof in a liquid or lyophilized composition and for increasing storage stability of such a composition are also provided.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 60/246,456, filed Nov. 7, 2000, and U.S. Provisional Application Serial No. 60/252,224, filed Nov. 21, 2000, each of which is hereby incorporated in its entirety by reference herein.

---------- KWIC ---------

Detail Description Paragraph - DETX (8):

[0032] The IFN-.beta. variants encompassed herein include muteins of the mature native IFN-.beta. sequence (see, for example, U.S. Pat. No.

5,814,485, herein incorporated by reference), wherein one or more **cysteine** residues that are not essential to biological activity have been deliberately deleted or replaced with other amino acids to eliminate sites for either intermolecular crosslinking or incorrect intramolecular disulfide bond formation. IFN-.beta., variants of this type include those containing a glycine, valine, alanine, leucine, isoleucine, tyrosine, phenylalanine, histidine, tryptophan, serine, threonine, or **methionine** substituted for the **cysteine** found at amino acid 17 of the mature native amino acid sequence. Serine and threonine are the more preferred replacements because of their chemical analogy to **cysteine**. Serine substitutions are most preferred. See, for example, the IFN-.beta. variant where the **cysteine** found at amino acid 17 of the mature native sequence is replaced with serine (U.S. Pat. No. 5,814,485). **Cysteine** 17 may also be deleted using methods known in the art (see, for example, U.S. Pat. No. 4,588,584, herein incorporated by reference), resulting in a mature IFN-.beta. mutein that is one amino acid shorter than the mature native IFN-.beta.. See also, as examples, U.S. Pat. Nos. 4,530,787; 4,572,798; and 4,588,585. Thus, IFN-.beta. variants with one or more **mutations** that improve, for example, their pharmaceutical utility are also encompassed by the present invention.

Detail Description Paragraph - DETX (28):

[0052] In addition to those agents disclosed above, other stabilizing agents, such as ethylenediaminetetracetic acid (EDTA) or one of its salts such as disodium EDTA, can be added to further enhance the stability of the liquid pharmaceutical compositions. The EDTA acts as a scavenger of metal ions known to catalyze many **oxidation reactions, thus providing an additional stabilizing** agent.

US-PAT-NO: 6576454

DOCUMENT-IDENTIFIER: US 6576454 B2

TITLE: Modified enzymes and their use for peptide synthesis

DATE-ISSUED: June 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Jones; J. Bryan	Lakefield	N/A	N/A	CA

APPL-NO: 10/ 075895

DATE FILED: February 13, 2002

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of Ser. No. 09/234,957, filed Jan. 21, 1999, pending, and claims the benefit of U.S. Provisional Patent Application No. 60/072,351, filed Jan. 23, 1998, abandoned, and U.S. Provisional Patent Application No. 60/072,265, filed Jan. 23, 1998. abandoned, the entire disclosures of which are hereby incorporated by reference in their entirety for all purposes.

US-CL-CURRENT: 435/222, 435/195 , 435/196 , 435/230 , 435/252.3 , 435/264 , 435/320.1 , 435/440 , 435/832 , 435/836 , 530/350 , 536/23.2

ABSTRACT:

The present invention relates to modified enzymes with one or more amino acid residues from an enzyme being replaced by cysteine residues, where at least some of the cysteine residues are modified by replacing thiol hydrogen in the cysteine residue with a thiol side chain to form a modified enzyme, wherein the modified enzyme has high esterase and low amidase activity. Also, a method of producing the modified enzymes is provided. The present invention also relates to a method for using the modified enzymes in peptide synthesis.

11 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Brief Summary Text - BSTX (7):

U.S. Pat. No. 5,208,158 to Bech et al. ("Bech") describes chemically modified detergent enzymes where one or more methionines have been mutated into cysteines. The cysteines are subsequently modified in order to confer upon the enzyme improved **stability towards oxidative** agents. The claimed chemical modification is the replacement of the thiol hydrogen with C.sub.1-6 alkyl.

Brief Summary Text - BSTX (8):

Although Bech has described altering the **oxidative stability** of an enzyme through mutagenesis and chemical modification, it would also be desirable to develop one or more enzymes with altered properties such as activity, nucleophile specificity, substrate specificity, stereoselectivity, thermal stability, pH activity profile, and surface binding properties for use in, for example, detergents or organic synthesis. In particular. enzymes, such as subtilisins, tailored for peptide synthesis would be desirable. Enzymes useful for peptide synthesis have high esterase and low amidase activities. Generally, subtilisins do not meet these requirements and the improvement of the esterase to amidase selectivities of subtilisins would be desirable. However, previous attempts to tailor enzymes for peptide synthesis by lowering amidase activity have generally resulted in dramatic decreases in both esterase and amidase activities. Previous strategies for lowering the amidase activity include the use of water-miscible organic solvents (Barbas et al., J. Am. Chem. Soc., 110:5162-5166 (1988); Wong et al., J. Am. Chem. Soc., 112:945-953 (1990); and Sears et al., Biotechnol. Prog., 12:423-433 (1996)) and site-directed mutagenesis (Abrahamsen et al., Biochemistry, 30:4151-4159 (1991); Bonneau et al., J. Am. Chem. Soc., 113:1026-1030 (1991); and Graycar et al., Ann. N.Y. Acad. Sci., 67:71-79 (1992)). However, while the ratios of esterase-to-amidase activities were improved by these approaches, the absolute esterase activities were lowered concomitantly. Abrahamsen et al., Biochemistry, 30:4151-4159 (1991). Chemical modification techniques (Neet et al., Proc. Nat. Acad. Sci., 56:1606 (1966); Polgar et al., J. Am. Chem. Soc., 88:3153-3154 (1966); Wu et al., J. Am. Chem. Soc., 111:4514-4515 (1989); and West et al., J. Am. Chem. Soc., 112:5313-5320 (1990)), which permit the incorporation of unnatural amino acid moieties, have also been applied to improve esterase to amidase selectivity of subtilisins. For example, chemical conversion of the catalytic triad serine (Ser221) of subtilisin to cysteine (Neet et al., Proc. Nat. Acad. Sci., 56:1606 (1966); Polgar et al., J. Am. Chem. Soc., 88:3153-3154 (1966); and Nakatsuka et al., J. Am. Chem. Soc., 109:3808-3810 (1987)) or to selenocysteine (Wu et al., J. Am. Chem. Soc., 111:4514-4515 (1989)), and methylation of the catalytic triad histidine (His57) of chymotrypsin (West et al., J. Am. Chem. Soc., 112:5313-5320 (1990)), effected substantial improvement in esterase-to-amidase selectivities. Unfortunately however, these modifications were again accompanied by 50-to 1000-fold decreases in absolute esterase activity.

Detailed Description Text - DETX (61):

At the Met222 site, both M222C--SCH.sub.2 CH.sub.2 NH.sub.3.sup.+ (-h) and M222C exhibited an improved esterase k.sub.cat /K.sub.M of up to 1.5, while all of M222C--S--CH.sub.3 (-a), M222C--SCH.sub.2 CH.sub.2 NH.sub.3.sup.+ (-h), and M222C displayed up to 37-fold reduced amidase activity. The

esterase-to-amidase activity of the **cysteine** parent, M222C, with its 4-fold improvement, was itself significantly higher than WT. The M222C **mutant** has a S.sub.1 ' leaving group site that is less sterically congested than WT. This may enhance the rate of acyl-enzyme hydrolysis, which is often the rate-determining step for ester substrates. M222C--S--CH.sub.3 (-a), which differs from WT only in the replacement of one of the **methionine** side-chain methylenes (CH.sub.2) by sulfur, had the same k.sub.cat as WT, but an increased K.sub.M. At this site, the most improved ME was M222C--SCH.sub.2 CH.sub.2 NH.sub.3.sup.+ (-h), which exhibited an esterase-to-amidase selectivity of 879, compared to 17 for the WT. This 52-fold improvement in esterase-to-amidase ratio of the series arose largely from a 31-fold lowered amidase k.sub.cat, but with the WT level of esterase k.sub.cat being retained. This result was consistent with the observation that the M222K **mutant** of subtilisin BPN' caused improved esterase activity and severely decreased amidase activity, thus, generating an enzyme with greatly improved esterase-to-amidase specificity. Graycar et al., Ann. N.Y. Acad. Sci., 672:71-79 (1992), which is hereby incorporated by reference.

US-PAT-NO: 6541207

DOCUMENT-IDENTIFIER: US 6541207 B1

TITLE: Methods for generating recombined polynucleotides

DATE-ISSUED: April 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vind; Jesper	V.ae butted.rl.o	N/A	N/A	DK
Borchert; Torben Vedel	slashed.se .O slashed.sterbro	N/A	N/A	DK

APPL-NO: 09/ 687301

DATE FILED: October 13, 2000

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a Rule 1.53(b) continuation application of U.S. patent application Ser. No. 09/040,697, filed Mar. 18, 1998, now U.S. Pat. No. 6,159,687 issued Dec. 12, 2000.

This application claims priority under 35 U.S.C. 119 of Danish applications 0307/97 filed Mar. 18, 1997, 0434/97 filed Apr. 17, 1997, and 0625/97 filed May 30, 1997, and U.S. Provisional applications Ser. Nos. 60/044,836, filed Apr. 25, 1997 and Ser. No. 60/153,012 filed Jun. 24, 1997, the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0307/97	March 18, 1997
DK	0625/97	May 30, 1997
DK	0434/97	April 17, 1997

US-CL-CURRENT: 435/6, 435/4 , 435/455 , 435/468 , 435/471 , 435/69.1 , 435/91.1 , 435/91.2 , 536/23.1

ABSTRACT:

A method for in vitro construction of a library of recombined homologous polynucleotides from a number of different starting DNA templates and primers by induced template shifts during an polynucleotide synthesis is described, whereby A. extended primers are synthesized by a) denaturing the DNA templates b) annealing primers to the templates, c) extending the said primers by use of a polymerase, d) stop the synthesis, and e) separate the extended primers from the templates, B. a template shift is induced by a) isolating the extended

primers from the templates and repeating steps A.b) to A.e) using the extended primers as both primers and templates, or b) repeating steps A.b) to A.e), C. this process is terminated after an appropriate number of cycles of process steps A. and B.a), A. and B.b), or combinations thereof.

Optionally the polynucleotides are amplified in a standard PCR reaction with specific primers to selectively amplify homologous polynucleotides of interest.

30 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (83):

Obviously the substitution of only one base within a codon doesn't provide total random **mutagenesis** (at protein level) as only a limited set of amino acid substitutions can be obtained by one base substitution at DNA level (e.g. **Methionine** encoded by ATG-codon requires three base substitution to become the TGT or TGC-codon encoding **Cysteine**).

Brief Summary Text - BSTX (98):

In the context of the present invention the term "positive polypeptide variants" means resulting polypeptide variants possessing functional properties which has been improved in comparison to the polypeptides producible from the corresponding input DNA sequences. Examples, of such improved properties can be as different as e.g. enhance or lowered biological activity, increased wash performance, thermostability, **oxidation stability,** substrate specificity, antibiotic resistance etc.

US-PAT-NO: 6506589

DOCUMENT-IDENTIFIER: US 6506589 B1

TITLE: Useful mutations of bacterial alkaline protease

DATE-ISSUED: January 14, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hastrup; Sven	K.o slashed.benhavn	N/A	N/A	DK
Branner; Sven	Lyngby	N/A	N/A	DK
Norris; Fanny	Hellerup	N/A	N/A	DK
Petersen; Steffen Bj.o	Ballerup	N/A	N/A	DK
slashed.rn	K.o slashed.ge	N/A	N/A	DK
N.o slashed.rskov-Lauridsen;	Bagsvaerd	N/A	N/A	DK
Leif	Roskilde	N/A	N/A	DK
Jensen; Villy Johannes				
Aaslyng; Dorrit				

APPL-NO: 08/ 486846

DATE FILED: June 7, 1995

PARENT-CASE:

This application is a divisional of U.S. application Ser. No. 07/294,241, filed Jan. 6, 1989 now abandoned, which is incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	00064/88	January 7, 1988

US-CL-CURRENT: 435/221, 435/220 , 435/222 , 435/252.3 , 435/252.31 , 435/320.1 , 435/471 , 435/69.1 , 510/300 , 536/23.2

ABSTRACT:

The present invention relates to mutations of the subtilisin gene, some of which result in changes in the chemical characteristics of subtilisin enzyme. Mutations are created at specific nucleic acids of the subtilisin gene and, in various specific embodiments, the mutant enzymes possess altered chemical properties including, but not limited to, increased **stability to oxidation,** augmented proteolytic activity, and improved washability. The present invention also relates, but is not limited to, the amino acid and DNA sequences for two proteases derived from Bacillus lentus variants, subtilisin 147 and subtilisin 309, as well as mutations of these genes and the corresponding mutant enzymes.

9 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

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Abstract Text - ABTX (1):
The present invention relates to mutations of the subtilisin gene, some of which result in changes in the chemical characteristics of subtilisin enzyme. Mutations are created at specific nucleic acids of the subtilisin gene and, in various specific embodiments, the mutant enzymes possess altered chemical properties including, but not limited to, increased **stability to oxidation,** augmented proteolytic activity, and improved washability. The present invention also relates, but is not limited to, the amino acid and DNA sequences for two proteases derived from Bacillus lentus variants, subtilisin 147 and subtilisin 309, as well as mutations of these genes and the corresponding mutant enzymes.

Brief Summary Text - BSTX (2):
The present invention relates to mutations of the subtilisin gene which result in changes in the chemical characteristics of subtilisin enzyme. Mutations at specific nucleic acids of the subtilisin gene result in amino acid substitutions and consequently, altered enzyme function. Some of these mutant enzymes exhibit physical properties advantageous to industrial applications, particularly in the detergent industry, providing subtilisin which is more **stable to oxidation,** possesses greater protease activity, and exhibits improved washability.

Brief Summary Text - BSTX (14):
The present invention relates to mutations of the subtilisin gene, some of which result in changes in the chemical characteristics of subtilisin enzyme. Mutations are created at specific nucleic acids of the subtilisin gene, and, in various specific embodiments, the mutant enzymes possess altered chemical properties including, but not limited to, increased **stability to oxidation,** augmented proteolytic ability, and improved washability.

Brief Summary Text - BSTX (16):
Site-directed mutation can efficiently produce mutant subtilisin enzymes which can be tailored to suit a multitude of industrial applications particularly in the areas of detergent and food technology. The present invention relates, in part, but is not limited to, mutants of the subtilisin 309 gene which exhibit improved **stability to oxidation,** augmented protease activity, and/or improved washability.

Detailed Description Text - DETX (3):
The invention is based, in part, upon the discovery that mutations of

specific nucleic acids in the subtilisin gene can result in enzymes with altered properties. In various embodiments, enzymes with improved **stability to oxidation,** augmented protease activity, or improved washing ability can be generated.

Detailed Description Text - DETX (38):
Once a variant of enhanced stability is identified by screening, the colony from which the variant is derived is isolated and the altered subtilisin is purified. Experiments can be performed on the purified enzyme to determine conditions of **stability towards oxidation,** thermal inactivation, denaturation temperature, kinetic parameters as well as other physical measurements. The altered gene can also be sequenced to determine the amino acid changes responsible for the enhanced stability. Using this procedure, variants with increased washing abilities have been isolated.

Detailed Description Text - DETX (63):
6.1.5. Determination of **Oxidation Stability**

Detailed Description Text - DETX (134):
6.2.4. **Oxidation Stability** of Mutant Subtilisins

Detailed Description Text - DETX (135):
The mutants a) and d) were tested for their **oxidation stability** in 0.01 M peracetic acid after 20 minutes at 50.degree. C. and pH 7. The parent strain NCIB 10309 protease was used as reference.

Detailed Description Text - DETX (137):
It is concluded that mutant d (Met 222 to Ala) exhibits superior **oxidation stability** realtive to the parent enzyme and mutant a.

Detailed Description Text - DETX (143):
From the table it is seen that mutant a) exhibits enhanced activity compared to the parent. It is also seen that the Met-222 mutants have lower activity than the parent, but due to their improved **oxidation stability** their application in detergent compositions containing oxidants is not precluded.

Detailed Description Text - DETX (151):
Subtilisin genes were cloned from the 147 and 309 variants of the bacterium Bacillus lentus, and the cloned genes were sequenced. By comparing the deduced amino acid sequences of subtilisins 147 and 309 one with the other and with sequences of other subtilisins, sites which, upon mutation, might alter the physical properties of the parent enzyme were identified. Site-directed mutagenesis was used to generate mutations at several of these sites in the subtilisin 309 gene. The resulting mutant enzymes were then expressed in a Bacillus strain, and tested against various physical and chemical parameters.

Several of the mutants were shown to have improved **stability to oxidation,** increased proteolytic ability, or improved washability when compared with parent subtilisin 309 enzyme. These mutants exhibit properties desirable in enzymes comprised in detergent compositions.

Detailed Description Paragraph Table - DETL (13):

TABLE IV **Oxidation Stability** Towards Peracetic Acid Residual Activity after 20 min. at 50.degree. C. Enzyme without oxidant with oxidant sub 309 89% 48% mutant a 83% 45% mutant d 92% 93%

Claims Text - CLTX (1):

1. A modified subtilisin 309, comprising one or more of the following **mutations**: (a) a substitution of the amino acid at position 153 with alanine; (b) a substitution of the amino acid at position 170 with tyrosine; and (c) a substitution of the amino acid at position 219 with **methionine or cysteine**; wherein each position corresponds to the position of the amino acid sequence of the mature subtilisin BPN' as depicted in Table I and the modified subtilisin has augmented protease activity or improved washing ability.

US-PAT-NO: 6406697

DOCUMENT-IDENTIFIER: US 6406697 B1

TITLE: Hybrid immunoglobulins

DATE-ISSUED: June 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Capon; Daniel J.	San Mateo	CA	N/A	N/A
Lasky; Laurence A.	Sausalito	CA	N/A	N/A

APPL-NO: 08/ 906549

DATE FILED: August 5, 1997

PARENT-CASE:

This application is a continuation of U.S. Ser. No. 08/451,848 filed May 26, 1995, now U.S. Pat. No. 5,714,147; which is a continuation of U.S. Ser. No. 08/185,670 filed Jan. 21, 1994, now U.S. Pat. No. 5,514,582; which is a continuation of U.S. Ser. No. 07/986,931 filed Dec. 8, 1992 now U.S. Pat. No. 5,428,130; which is a continuation of U.S. Ser. No. 07/808,122; filed Dec. 16, 1991, now U.S. Pat. No. 5,225,538; which is a divisional of U.S. Ser. No. 07/440,625 filed Nov. 22, 1989, now U.S. Pat. No. 5,116,964; which is a continuation-in-part of U.S. Ser. No. 07/315,015 filed Feb. 23, 1989, now U.S. Pat. No. 5,098,833, to which the present application claims the benefit of priority under 35 U.S.C. .sctn.120.

US-CL-CURRENT: 424/178.1, 435/69.7 , 514/2 , 530/350 , 536/23.4

ABSTRACT:

Novel polypeptides are provided, together with methods for making and using them, and nucleic acids encoding them. These polypeptides are useful as cell surface adhesion molecules and ligands, and are useful in therapeutic or diagnostic compositions and methods.

4 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 16

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Detailed Description Text - DETX (78):

Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **xidative stability** of the LHR. Deletion or substitutions of potential proteolysis sites, e.g. Arg Arg, is accomplished by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

Detailed Description Text - DETX (223):

Correct **mutants** were tested for expression by transfection onto human kidney 293 cells using previously described methods. 35S **methionine and cysteine** labeled supernatants were analyzed by immunoprecipitation with protein A sepharose beads in the absence of added antibodies. The precipitated proteins were analyzed on 7.5% polyacrylamide-SDS gels either with or without reduction with beta mercaptoethanol. Plasmids that resulted in correctly expressed chimeras were introduced into 293 cells by transfection in the presence of selective plasmids encoding resistance to G418 as well as dihydrofolate reductase. Clones were selected in G418, and the incorporated plasmids were amplified in the presence of methotrexate. Permanent cell lines expressing high levels of each construct were grown to large scale in T-flasks, and the cell supernatants were clarified by centrifugation and filtration. The resultant supernatants were concentrated by Amicon filtration and passed over standard protein A-sepharose columns, washed with PBS, and eluted with 0.1M Acetic Acid, 0.15 M NaCl (pH 3.5). The eluted material was immediately neutralized with 3 M Tris, pH 9, and quantitated by SDS gel electrophoresis as well as an ELISA assay.

US-PAT-NO: 6403331

DOCUMENT-IDENTIFIER: US 6403331 B1

TITLE: Mutant proteolytic enzymes and method of production

DATE-ISSUED: June 11, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Christianson; Teresa	Cotati	CA	N/A	N/A
Goddette; Dean	Rohnert Park	CA	N/A	N/A
Ladin; Beth Frances	Santa Rosa	CA	N/A	N/A
Lau; Maria R.	Fairfield	CA	N/A	N/A
Paech; Christian	Santa Rosa	CA	N/A	N/A
Reynolds; Robert B.	Santa Rosa	CA	N/A	N/A
Wilson; Charles R.	Santa Rosa	CA	N/A	N/A
Yang; Shiow-Shong	Santa Rosa	CA	N/A	N/A

APPL-NO: 09/ 585798

DATE FILED: May 31, 2000

PARENT-CASE:

This is a continuation application of application Ser. No. 08/980,135, filed Nov. 26, 1997 now U.S. Pat. No. 6,136,553 which was a divisional application of application Ser. No. 08/618,446, filed Mar. 19, 1996, now U.S. Pat. No. 5,985,639 which was a divisional application of application Ser. No. 08/254,021, filed Jun. 2, 1994, now U.S. Pat. No. 5,500,364, which was a divisional of application Ser. No. 07/706,691, filed May 29, 1991, now U.S. Pat. No. 5,340,735.

US-CL-CURRENT: 435/23, 435/219 , 435/220 , 435/221 , 435/24 , 702/19

ABSTRACT:

Mutant Bacillus lentus DSM 5483 proteases are derived by the replacement of at least one amino acid residue of the mature form of the B. lentus DSM 5483 alkaline protease. The mutant proteases are expressed by genes which are mutated by site-specific mutagenesis. The amino acid sites selected for replacement are identified by means of a computer based method which compares the three dimensional structure of the wild-type protease and a reference protease.

23 Claims, 18 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

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Brief Summary Text - BSTX (5):

Subtilisins are a family of extracellular proteins having molecular weights in the range of 25,000-35,000 daltons and are produced by various Bacillus species. These proteins function as peptide hydrolases in that they catalyze the hydrolysis of peptide linkages in protein substrates at neutral and alkaline pH values. Subtilisins are termed serine proteases because they contain a specific serine residue which participates in the catalytic hydrolysis of peptide substrates. A subtilisin enzyme isolated from soil samples and produced by Bacillus lentus for use in detergent formulations having increased protease and **oxidative stability** over commercially available enzymes under conditions of pH 7 to 10 and at temperature of 10 to 60.degree. C. in aqueous solutions has been disclosed in copending patent application Ser. No. 07/398,854, filed on Aug. 25, 1989. This B. lentus alkaline protease enzyme (BLAP, vide infra) is obtained in commercial quantities by cultivating a Bacillus licheniformis ATCC 53926 strain which had been transformed by an expression plasmid which contained the wild type BLAP gene and the B. licheniformis ATCC 53926 alkaline protease gene promoter.

Brief Summary Text - BSTX (6):

Industrial processes generally are performed under physical conditions which require highly stable enzymes. Enzymes may be inactivated by high temperatures, pH extremes, oxidation, and surfactants. Even though Bacillus subtilisin proteases are currently used in many industrial applications, including detergent formulations, stability improvements are still needed. Market trends are toward more concentrated detergent powders, and an increase in liquid formulations. Increased shelf **stability and oxidative stability,** with retention of catalytic efficiency are needed. It is therefore desirable to isolate novel enzymes with increased stability, or to improve the stability of existing enzymes, including subtilisin proteases such as BLAP.

Brief Summary Text - BSTX (14):

EP 0251446 teaches the construction of mutant carbonyl hydrolases (proteases) which have at least one property different from the parental carbonyl hydrolase. It describes mutations which effect (either improve or decrease) **oxidative stability,** substrate specificity, catalytic activity, thermal stability, alkaline stability, pH activity profile, and resistance to autoproteolysis. These mutations were selected for introduction into Bacillus amyloliguefaciens subtilisin BPN' after alignment of the primary sequences of BPN' and proteases from B. subtilis, B. licheniformis, and thermitase. Such alignment can then be used to select amino acids in these other proteases which differ, as substitutes for the equivalent amino acid in the B. amyloliquefaciens carbonyl hydrolase. This application also describes alignment on the basis of a 1.8 .ANG. X-ray crystal structure of the B. amyloliquefaciens protease. Amino acids in the carbonyl hydrolase of B. amyloliguefaciens which when altered can affect stability, substrate specificity, or catalytic efficiency include: Met50, Met124, and Met222 for

oxidative stability; Tyr104, Ala152, Glu156, Gly166, Gly169, Phe189, and Tyr217 for substrate specificity; N155 alterations were found to decrease turnover, and lower Km; Asp36, Ile107, Lys170, Asp197, Ser204, Lys213, and Met222 for alkaline stability; and Met199, and Tyr21 for thermal stability. Alteration of other amino acids was found to affect multiple properties of the protease. Included in this category are Ser24, Met50, Asp156, Gly166, Gly169, and Tyr217. Substitution at residues Ser24, Met50, Ile107, Glu156, Gly166, Gly169, Ser204, Lys213, Gly215, and Tyr217 was predicted to increase thermal and alkaline stability. An important point about this patent application is that with the exception of those mutations effecting substrate specificity, no rational mutational approach for improving the alkaline or temperature stability of a protease based upon computer simulations of an X-ray crystal structure is described.

Brief Summary Text - BSTX (18):

Sensitivity to oxidation is an important deficiency of serine proteases used in detergent applications (Stauffer, C. E., and Etson, D. (1969) J. Biol. Chem. 244:5333-5338). EP 0130756, EP 0247647, and U.S. Pat. No. 4,760,025 teach a saturation **mutation** method where one or multiple **mutations** are introduced into the subtilisin BPN' at amino acid residues Asp32, Asn155, Tyr104, Met222, Gly166, His64, Ser221, Gly169, Glu156, Ser33, Phe189, Tyr217, and/or Ala152. Using this approach **mutant** proteases exhibiting improved **oxidative stability,** altered substrate specificity, and/or altered pH activity profiles are obtained. A method is taught in which improved **oxidative stability** is achieved by substitution of **methionine, cysteine,** tryptophan, and lysine residues. These publications also teach that **mutations** within the active site region of the protease are also most likely to influence activity. Random or selected **mutations** can be introduced into a target gene using the experimental approach but neither EP 0130756, EP 0247647, nor U.S. Pat. No. 4,760,025 teach a method for predicting amino acid alterations which will improve the thermal or surfactant stability of the protease.

US-PAT-NO: 6395532

DOCUMENT-IDENTIFIER: US 6395532 B1

TITLE: Modified enzymes and their use for peptide synthesis

DATE-ISSUED: May 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Jones; J. Bryan	Lakefield, Ontario	N/A	N/A	CA

APPL-NO: 09/ 234957

DATE FILED: January 21, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Ser. No. 60/072,351, filed Jan. 23, 1998, and U.S. Provisional Patent Application Ser. No. 60/072,265, filed Jan. 23, 1998, and which are hereby incorporated by reference.

US-CL-CURRENT: 435/222, 435/195 , 435/196 , 435/230 , 435/252.3 , 435/264 , 435/320.1 , 435/440 , 435/832 , 435/836 , 530/350 , 536/23.2

ABSTRACT:

The present invention relates to modified enzymes with one or more amino acid residues from an enzyme being replaced by cysteine residues, where at least some of the cysteine residues are modified by replacing thiol hydrogen in the cysteine residue with a thiol side chain to form a modified enzyme, wherein the modified enzyme has high esterase and low amidase activity. Also, a method of producing the modified enzymes is provided. The present invention also relates to a method for using the modified enzymes in peptide synthesis.

19 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

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Brief Summary Text - BSTX (7):

U.S. Pat. No. 5,208,158 to Bech et al. ("Bech") describes chemically modified detergent enzymes where one or more methionines have been mutated into cysteines. The cysteines are subsequently modified in order to confer upon the enzyme improved **stability towards oxidative** agents. The claimed chemical modification is the replacement of the thiol hydrogen with C.sub.1-6 alkyl.

Brief Summary Text - BSTX (8):

Although Bech has described altering the **oxidative stability** of an enzyme through mutagenesis and chemical modification, it would also be desirable to develop one or more enzymes with altered properties such as activity, nucleophile specificity, substrate specificity, stereoselectivity, thermal stability, pH activity profile, and surface binding properties for use in, for example, detergents or organic synthesis. In particular, enzymes, such as subtilisins, tailored for peptide synthesis would be desirable. Enzymes useful for peptide synthesis have high esterase and low amidase activities. Generally, subtilisins do not meet these requirements and the improvement of the esterase to amidase selectivities of subtilisins would be desirable. However, previous attempts to tailor enzymes for peptide synthesis by lowering amidase activity have generally resulted in dramatic decreases in both esterase and amidase activities. Previous strategies for lowering the amidase activity include the use of water-miscible organic solvents (Barbas et al., J. Am. Chem. Soc., 110:5162-5166 (1988); Wong et al., J. Am. Chem. Soc., 112:945-953 (1990); and Sears et al., Biotechnol. Prog., 12:423-433 (1996)) and site-directed mutagenesis (Abrahamsen et al., Biochemistry, 30:4151-4159 (1991); Bonneau et al., J. Am. Chem. Soc., 113:1026-1030 (1991); and Graycar et al., Ann. N. Y. Acad. Sci., 67:71-79 (1992)). However, while the ratios of esterase-to-amidase activities were improved by these approaches, the absolute esterase activities were lowered concomitantly. Abrahamsen et al., Biochemistry, 30:4151-4159 (1991). Chemical modification techniques (Neet et al., Proc. Nat. Acad. Sci., 56:1606 (1966); Polgar et al., J. Am. Chem. Soc., 88:3153-3154 (1966); Wu et al., J. Am. Chem. Soc., 111:4514-4515 (1989); and West et al., J. Am. Chem. Soc., 112:5313-5320 (1990)), which permit the incorporation of unnatural amino acid moieties, have also been applied to improve esterase to amidase selectivity of subtilisins. For example, chemical conversion of the catalytic triad serine (Ser221) of subtilisin to cysteine (Neet et al., Proc. Nat. Acad. Sci., 56:1606 (1966); Polgar et al., J. Am. Chem. Soc., 88:3153-3154 (1966); and Nakatsuka et al., J. Am. Chem. Soc., 109:3808-3810 (1987)) or to selenocysteine (Wu et al., J. Am. Chem. Soc., 111:4514-4515 (1989)), and methylation of the catalytic triad histidine (His57) of chymotrypsin (West et al., J. Am. Chem. Soc., 112:5313-5320 (1990)), effected substantial improvement in esterase-to-amidase selectivities. Unfortunately however, these modifications were again accompanied by 50- to 1000-fold decreases in absolute esterase activity.

Detailed Description Text - DETX (72):

At the Met222 site, both M222C--SCH.sub.2 CH.sub.2 NH.sub.3.sup.+ (-h) and M222C exhibited an improved esterase k.sub.cat /K.sub.M of up to 1.5, while all of M222C--S--CH.sub.3 (-a), M222C--SCH.sub.2 CH.sub.2 NH.sub.3.sup.+ (-h), and M222C displayed up to 37-fold reduced amidase activity. The esterase-to-amidase activity of the **cysteine** parent, M222C, with its 4-fold improvement, was itself significantly higher than WT. The M222C **mutant** has a

S.sub.1 ' leaving group site that is less sterically congested than WT. This may enhance the rate of acyl-enzyme hydrolysis, which is often the rate-determining step for ester substrates. M222C--S--CH.sub.3 (-a), which differs from WT only in the replacement of one of the **methionine** side-chain mehtylenes (CH.sub.2) by sulfur, had the same k.sub.cat as WT, but an increased K.sub.M. At this site, the most improved ME was M222C--SCH.sub.2 CH.sub.2 NH.sub.3.sup.+ (-h), which exhibited an esterase-to-amidase selectivity of 879, compared to 17 for the WT. This 52-fold improvement in esterase-to-amidase ratio of the series arose largely from a 31-fold lowered amidase k.sub.cat, but with the WT level of esterase k.sub.cat being retained. This result was consistent with the observation that the M222K **mutant** of subtilisin BPN' caused improved esterase activity and severely decreased amidase activity, thus, generating an enzyme with greatly improved esterase-to-amidase specificity. Graycar et al., Ann. N.Y. Acad. Sci., 672:71-79 (1992), which is hereby incorporated by reference.

US-PAT-NO: 6361974

DOCUMENT-IDENTIFIER: US 6361974 B1
See image for Certificate of Correction

TITLE: Exonuclease-mediated nucleic acid reassembly in directed evolution

DATE-ISSUED: March 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Short; Jay M.	Rancho Santa Fe	CA	N/A	N/A
Djavakhishvili; Tsotne David	San Diego	CA	N/A	N/A
Frey; Gerhard Johann	San Diego	CA	N/A	N/A

US-CL-CURRENT: 435/69.1, 530/350 , 536/23.2

ABSTRACT:

This invention provides methods of obtaining novel polynucleotides and encoded polypeptides by the use of non-stochastic methods of directed evolution (DirectEvolution.TM.). A particular advantage of exonuclease-mediated reassembly methods is the ability to reassemble nucleic acid strands that would otherwise be problematic to chimerize. Exonuclease-mediated reassembly methods can be used in combination with other mutagenesis methods provided herein. These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). This invention provides methods of obtaining novel enzymes that have optimized physical &/or biological properties. Through use of the claimed methods, genetic vaccines, enzymes, small molecules, and other desirable molecules can be evolved towards desirable properties. For example, vaccine vectors can be obtained that exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like. Furthermore, this invention provides methods of obtaining a variety of novel biologically active molecules, in the fields of antibiotics, pharmacotherapeutics, and transgenic traits.

15 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

US-PAT-NO: 6358709

DOCUMENT-IDENTIFIER: US 6358709 B1

TITLE: End selection in directed evolution

DATE-ISSUED: March 19, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Short; Jay M.	Encinitas	CA	N/A	N/A
Frey; Gerhard Johann	San Diego	CA	N/A	N/A

US-CL-CURRENT: 435/69.1, 530/350 , 536/23.2

ABSTRACT:

This invention provides methods of obtaining novel polynucleotides and encoded polypeptides by the use of non-stochastic methods of directed evolution (DirectEvolution.TM.). A particular advantage of end-selection-based methods is the ability to recover full-length polynucleotides from a library of progeny molecules generated by mutagenesis methods. These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). This invention provides methods of obtaining novel enzymes that have optimized physical &/or biological properties. Through use of the claimed methods, genetic vaccines, enzymes, small molecules, and other desirable molecules can be evolved towards desirable properties. For example, vaccine vectors can be obtained that exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like. Furthermore, this invention provides methods of obtaining a variety of novel biologically active molecules, in the fields of antibiotics, pharmacotherapeutics, and transgenic traits.

36 Claims, 11 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

US-PAT-NO: 6297037

DOCUMENT-IDENTIFIER: US 6297037 B1
See image for Certificate of Correction

TITLE: **Oxidatively stable** alpha-amylase

DATE-ISSUED: October 2, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barnett; Christopher C.	South San Francisco	CA	94080	N/A
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Power; Scott D.	San Bruno	CA	94066	N/A
Requadt; Carol A.	Tiburon	CA	94920	N/A

APPL-NO: 08/ 194664

DATE FILED: February 10, 1994

PARENT-CASE:

RELATED APPLICATIONS

This application is a continuation-in-part of U.S. Ser. No. 08/016,395 filed Feb. 11, 1993, now abandoned.

US-CL-CURRENT: 435/202, 435/201 , 435/203 , 435/274 , 435/275 , 435/471 , 435/485 , 510/226 , 510/320 , 510/392 , 510/393

ABSTRACT:

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such mutant alpha-amylases have altered **oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

25 Claims, 26 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

---------- KWIC ---------

Abstract Text - ABTX (1):

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such mutant alpha-amylases have altered **oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

TITLE - TI (1):

Oxidatively stable alpha-amylase

Brief Summary Text - BSTX (2):

The present invention relates to novel alpha-amylase mutants having an amino acid sequence not found in nature, such mutants having an amino acid sequence wherein one or more amino acid residue(s) of a precursor alpha-amylase, specifically any oxidizable amino acid, have been substituted with a different amino acid. The mutant enzymes of the present invention exhibit altered stability/activity profiles including but not limited to altered **oxidative stability,** altered pH performance profile, altered specific activity and/or altered thermostability.

Brief Summary Text - BSTX (7):

In order to alter the stability or activity profile of amylase enzymes under varying conditions, it has been found that selective replacement, substitution or deletion of oxidizable amino acids, such as a **methionine,** tryptophan, tyrosine, histidine or **cysteine,** results in an altered profile of the variant enzyme as compared to its precursor. Because currently commercially available amylases are not acceptable (stable) under various conditions, there is a need for an amylase having an altered stability and/or activity profile. This altered **stability (oxidative,** thermal or pH performance profile) can be achieved while maintaining adequate enzymatic activity, as compared to the wild-type or precursor enzyme. The characteristic affected by introducing such **mutations** may be a change in **oxidative stability** while maintaining thermal stability or vice versa. Additionally, the substitution of different amino acids for an oxidizable amino acids in the alpha-amylase precursor sequence or the deletion of one or more oxidizable amino acid(s) may result in altered enzymatic activity at a pH other than that which is considered optimal for the precursor alpha-amylase. In other words, the **mutant** enzymes of the present invention may also have altered pH performance profiles, which may be due to the enhanced **xidative stability** of the enzyme.

Brief Summary Text - BSTX (10):

Preferably the substitution or deletion of one or more amino acid in the amino acid sequence is due to the replacement or deletion of one or more methionine, tryptophan, cysteine, histidine or tyrosine residues in such sequence, most preferably the residue which is changed is a methionine residue. The oxidizable amino acid residues may be replaced by any of the other 20 naturally occurring amino acids. If the desired effect is to alter the **oxidative stability** of the precursor, the amino acid residue may be substituted with a non-oxidizable amino acid (such as alanine, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, or valine) or another oxidizable amino acid (such as cysteine, methionine, tryptophan, tyrosine or histidine, listed in order of most easily oxidizable to less readily oxidizable). Likewise, if the desired effect is to alter thermostability, any of the other 20 naturally occurring amino acids may be substituted (i.e., cysteine may be substituted for methionine).

Brief Summary Text - BSTX (11):

Preferred **mutants** comprise the substitution of a **methionine** residue equivalent to any of the **methionine** residues found in B. licheniformis alpha-amylase (+8, +15, +197, +256, +304, +366 and +438). Most preferably the **methionine** to be replaced is a **methionine** at a position equivalent to position +197 or +15 in B. licheniformis alpha-amylase. Preferred substitute amino acids to replace the **methionine** at position +197 are alanine (A), isoleucine (I), threonine (T) or **cysteine** (C). The preferred substitute amino acids at position +15 are leucine (L), threonine (T), asparagine (N), aspartate (D), serine (S), valine (V) and isoleucine (I), although other substitute amino acids not specified above may be useful. Two specifically preferred **mutants** of the present invention are M197T and M15L.

Brief Summary Text - BSTX (13):

The alpha-amylase mutants of the present invention, in general, exhibit altered **oxidative stability** in the presence of hydrogen peroxide and other oxidants such as bleach or peracids, or, more specific, milder oxidants such as chloramine-T. Mutant enzymes having enhanced **oxidative stability** will be useful in extending the shelf life and bleach, perborate, percarbonate or peracid compatibility of amylases used in cleaning products. Similarly, reduced **oxidative stability** may be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity. The mutant enzymes of the present invention may also demonstrate a broadened pH performance profile whereby mutants such as M15L show stability for low pH starch liquefaction and mutants such as M197T show stability at high pH cleaning product conditions. The mutants of the present invention may also have altered thermal stability whereby the mutant may have enhanced stability at either high or low temperatures. It is understood that any change (increase or decrease) in the mutant's enzymatic characteristic(s), as compared to its precursor, may be beneficial depending on the desired end use of the mutant alpha-amylase .

Detailed Description Text - DETX (2):

It is believed that amylases used in starch liquefaction may be subject to some form of inactivation due to some activity present in the starch slurry (see commonly owned U.S. applications Ser. Nos. 07/785,624 and 07/785,623 and U.S. Pat. No. 5,180,669, issued Jan. 19, 1993, incorporated herein by reference). Furthermore, use of an amylase in the presence of oxidants, such as in bleach or peracid containing detergents, may result in partial or complete inactivation of the amylase. Therefore, the present invention focuses on altering the oxidative sensitivity of amylases. The mutant enzymes of the present invention may also have an altered pH profile and/or altered thermal **stability which may be due to the enhanced oxidative stability** of the enzyme at low or high pH's.

Detailed Description Text - DETX (7):

Specific residues corresponding to positions M197, M15 and W138 of Bacillus licheniformis alpha-amylase are identified herein for substitution or deletion, as are all **methionine,** histidine, tryptophan, **cysteine** and tyrosine positions. The amino acid position number (i.e., +197) refers to the number assigned to the mature Bacillus licheniformis alpha-amylase sequence presented in FIG. 2. The invention, however, is not limited to the **mutation** of this particular mature alpha-amylase (B. licheniformis) but extends to precursor alpha-amylases containing amino acid residues at positions which are equivalent to the particular identified residue in B. licheniformis alpha-amylase. A residue (amino acid) of a precursor alpha-amylase is equivalent to a residue of B. licheniformis alpha-amylase if it is either homologous (i.e., corresponding in position in either primary or tertiary structure) or analogous to a specific residue or portion of that residue in B. licheniformis alpha-amylase (i.e., having the same or similar functional capacity to combine, react, or interact chemically or structurally).

Detailed Description Text - DETX (14):

Based on the conditions of a preferred liquefaction process, as described in commonly owned U.S. applications Ser. Nos. 07/788,624 and 07/785,623 and U.S. Pat. No. 5,180,669, namely low pH, high temperature and potential oxidation conditions, preferred mutants of the present invention for use in liquefaction processes comprise mutants exhibiting altered pH performance profiles (i.e., low pH profile, pH <6 and preferably pH <5.5), and/or altered thermal stability (i.e., high temperature, about 90.degree.-110.degree. C.), and/or altered **oxidative stability (i.e., enhanced oxidative stability)**.

Detailed Description Text - DETX (78):

As can be As can be seen in FIG. 9, there was a wide range of amylase activity (measured in the soluble substrate assay) expressed by the M197X (A4 form) variants. The amylases were partially purified from the supernatants by precipitation with two volumes of ethanol and resuspension. They were then screened for thermal stability (FIG. 10) by heating at 95.degree. C. for 5 minutes in 10 mM acetate buffer pH 5.0, in the presence of 5 mM calcium chloride; the A4 wild-type retained 28% of its activity after incubation. For M197W and M197P we were unable to recover active protein from the supernatants.

Upon sequencing, the M197H variant was found to contain a second mutation, N190K. M197L was examined in a separate experiment and was one of the lowest thermally stable variants. There appears to be a broad correlation between expression of amylase activity and thermal stability. The licheniformis amylase is restricted in what residues it can accommodate at position 197 in terms of retaining or enhancing thermal stability: cysteine and threonine are preferred for maximal thermal stability under these conditions whereas alanine and isoleucine are of intermediate stability. However, other substitutions at position +197 result in lowered thermal stability which may be useful for other applications. Additionally, different substitutions at +197 may have other beneficial properties, such as altered pH performance profile or altered **oxidative stability**. For example, the M197C variant was found to inactivate readily by air **oxidation but had enhanced thermal stability**. Conversely, compared to the M197L variant, both M197T and M197A retained not only high thermal stability (FIG. 10), but also high activity (Table III), while maintaining resistance to inactivation by peroxide at pH 5 to pH 10 (FIG. 7).

Claims Text - CLTX (24):

24. A mutant alpha-amylase having enhanced **oxidative stability,** the mutant alpha-amylase being derived from Bacillus and comprising a substitution of methionine with alanine, arginine, glycine, lysine, phenylalanine, proline, threonine or valine at an amino acid residue equivalent to M197 in Bacillus licheniformis alpha-amylase.

US-PAT-NO: 6211134

DOCUMENT-IDENTIFIER: US 6211134 B1
See image for Certificate of Correction

TITLE: Mutant .alpha.-amylase

DATE-ISSUED: April 3, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Caldwell; Robert M.	San Carlos	CA	N/A	N/A
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Ropp; Traci H	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 985659

DATE FILED: December 9, 1997

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation in part of U.S. Ser. No. 08/645,971 filed on May 14, 1996 U.S. Pat. No. 5,763,385.

US-CL-CURRENT: 510/392, 510/226 , 510/321 , 510/330

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed having a substution equivalent to G475R in Bacillus licheniformis. The disclosed .alpha.-amylase enzymes show improved specific activity and starch hydrolysis performance. Also provided are polynucleotides encoding such enzymes, expression vectors including such polynucleotides, host cells transformed with such expression vectors, and the use of such enzymes in detergent compositions.

17 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

---------- KWIC ---------

Brief Summary Text - BSTX (16):

In PCT Publication No. WO 94/02597, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more methionines are replaced

by any amino acid except **cysteine or methionine**.

Brief Summary Text - BSTX (17):

In PCT publication No. WO 94/18314, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more of the **methionine,** tryptophan, **cysteine,** histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Detailed Description Text - DETX (13):

The .alpha.-amylases according to the present invention exhibit improved specific activity and liquefaction performance providing desirable and unexpected results which are useful in the various applications for which .alpha.-amylases are commonly used. The .alpha.-amylase of the present invention is especially useful in starch processing and particularly in starch liquefaction. Conditions present during commercially desirable liquefaction processes characteristically include low pH, high temperature and potential oxidation conditions requiring .alpha.-amylases exhibiting improved low pH performance, improved thermal **stability and improved oxidative stability**. Accordingly, .alpha.-amylases according to the present invention which are particularly useful in liquefaction exhibit improved performance at a pH of less than about 6, preferably less than about 5.5, and most preferably between about 5.0 and 5.5. Additionally, .alpha.-amylases according to the present invention which exhibit increased thermal stability at temperatures of between about 80-120.degree. C., and preferably between about 100-110.degree. C., and increased stability in the presence of oxidants will be particularly useful. Preferably, the .alpha.-amylase according to the present invention which is used in liquefaction, in addition to substitution of a residue corresponding to G475, further comprises a deletion or substitution at one or more residues corresponding to M15, V128, H133, W138, V148, S187, M197, A209 and/or A379 in Bacillus licheniformis. Most preferably, the amylase comprises a substitution corresponding to M15T/H133Y/V148S/N188SA209V/A379S/G475R in Bacillus licheniformis. In any event, because it is contemplated that many mutations provide incremental advantages, the combination of such a mutation with the mutants of the invention should provide additive benefits. Thus, for example, because a mutation corresponding to M197T has been established as providing exceptional **oxidation stability,** the addition of a M197T modification to a mutant .alpha.-amylase of the invention should provide a similar boost in **oxidative stability**.

Detailed Description Text - DETX (16):

In another embodiment of the present invention there are provided detergent compositions in either liquid, gel or granular form, which comprise the .alpha.-amylase according to the present invention. Such detergent compositions will particularly benefit from the addition of an .alpha.-amylase according to the present invention which has increased thermal stability to improve shelf-life or increased **oxidative stability** such that the .alpha.-amylase has improved resistance to bleach or peracid compounds commonly present in detergents. Thus, .alpha.-amylase according to the present invention may be advantageously formulated into known powdered, liquid or gel detergents having a pH of between about 6.5 and about 12.0. A preferred

embodiment of the present invention further comprises the deletion or substitution of a methionine residue or a tryptophan residue, for example M15, M197 or W138 as described in commonly assigned U.S. patent application Ser. Nos. 08/289,351 and 08/409,771, the disclosures of which are incorporated by reference; substitution at M133Y as described in PCT Publication No. WO 91/00353; or substitution at A209 as described in DeClerck, et al., J. Biol. Chem., Vol. 265, pp. 15481-15488 (1990). Also preferably, an .alpha.-amylase according to the present invention used in detergent compositions. Detergent compositions comprising the .alpha.-amylase according to the present invention may further include other enzymes such as endoglycosidases, cellulases, proteases, lipases or other amylase enzymes, particularly .alpha.-amylase derived from Bacillis stearothermophilus, as well as additional ingredients as generally known in the art.

Detailed Description Text - DETX (17):

Embodiments of the present invention which comprise a combination of the .alpha.-amylase according to the present invention with protease enzymes preferably include **oxidatively stable** proteases such as those described in U.S. Pat. No. Re. 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk), MAXAPEM (Gist-brocades) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease mutants (**oxidatively stable** proteases), and particularly such mutants having a substitution for the methionine at a position equivalent to M222 in Bacillus amyloliquefaciens, are described in U.S. Pat. No. Re. 34,606.

Detailed Description Text - DETX (20):

The improved .alpha.-amylases according to the present invention provide several important advantages when compared to wild type Bacillus .alpha.-amylases. For example, one advantage is the increased activity found at low pH and high temperatures typical of common starch liquefaction methods. Another advantage is the increased high pH and **oxidative stability** which facilitates their use in detergents. Another advantage is that a more complete hydrolysis of starch molecules is achieved which reduces residual starch in the processing stream. Yet another advantage is their improved stability in the absence of calcium ion. Yet another advantage is that the addition of equal protein doses of .alpha.-amylase according to the invention provide superior performance when compared to wild type Bacillus licheniformis .alpha.-amylase due to improvements in both specific activity and stability under stressed conditions. In other words, because of the generally increased stability of the amylases according to the present invention, the increased specific activity on starch of the inventive amylases translates to even greater potential performance benefits of this variant. Under conditions where the wild type enzyme is being inactivated, not only does more of the inventive amylase survive because of its increased stability, but also that which does survive expresses proportionally more activity because of its increased specific activity.

US-PAT-NO: 6184002

DOCUMENT-IDENTIFIER: US 6184002 B1

TITLE: Method for liquefying starch

DATE-ISSUED: February 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A

APPL-NO: 08/ 952225

DATE FILED: January 27, 1998

PCT-DATA:
APPL-NO: PCT/US96/08144
DATE-FILED: May 30, 1996
PUB-NO: WO96/38578
PUB-DATE: Dec 5, 1996
371-DATE: Jan 27, 1998
102(E)-DATE:Jan 27, 1998

US-CL-CURRENT: 435/99, 435/201

ABSTRACT:

According to the invention a method is provided for liquefying starch comprising the steps of adding a sodium composition to the starch prior to or simultaneously with liquefying the starch; adding .alpha.-amylase to the treated starch; and reacting the treated starch for a time and at a temperature effective to liquefy the treated starch. Preferred sodium compositions comprise sodium chloride, sodium bicarbonate, sodium benzoate, sodium sulfate, sodium bisulfite, sodium ascorbate, sodium acetate, sodium nitrate, sodium tartrate, sodium tetraborate, sodium propionate, sodium citrate, sodium succinate, monosodium glutamate, trisodium citrate, sodium phosphate or a mixture thereof.

14 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (14):
In U.S. Pat. No. 5,180,669, liquefaction between a pH of 5.0 to 6.0 was

achieved by the addition of carbonate ion in excess of the amount needed to buffer the solution to the ground starch slurry. Due to an increased pH effect which occurs with addition of carbonate ion, the slurry is generally neutralized by adding a source of hydrogen ion, for example, an inorganic acid such as hydrochloric acid or sulfuric acid. In PCT Publication No. WO 94/02597, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more methionines are replaced by any amino acid except **cysteine or methionine**.

Brief Summary Text - BSTX (15):

In PCT publication No. 94/18314, a **mutant** -amylase having improved **oxidative stability** is described wherein one or more of the **methionine,** tryptophan, **cysteine,** histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

US-PAT-NO: 6171826

DOCUMENT-IDENTIFIER: US 6171826 B1

TITLE: Methods of controlling beta dimer formation in hemoglobin

DATE-ISSUED: January 9, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Levine; Joseph D.	Louisville	CO	N/A	N/A
Apostol; Izydor A.	Boulder	CO	N/A	N/A

APPL-NO: 09/ 230603

DATE FILED: May 14, 1999

PARENT-CASE:

This appln is a 371 of PCT/US97/13564 filed Aug. 1, 1997 and also claims the benefit of U.S. Provisional No. 60/023,211 filed Aug. 2, 1996.

PCT-DATA:
APPL-NO: PCT/US97/13564
DATE-FILED: August 1, 1997
PUB-NO: WO98/05773
PUB-DATE: Feb 12, 1998
371-DATE: May 14, 1999
102(E)-DATE:May 14, 1999

US-CL-CURRENT: 435/69.6, 530/385

ABSTRACT:

The present invention relates to methods of controlling beta dimer formation in hemoglobin solutions by altering the metal binding site adjacent to the N-terminus of beta globins. The invention further relates to methods of producing stable, intramolecularly crosslinked beta globins by exposure to Ni(II) and oxone.

13 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

---------- KWIC ---------

Detailed Description Text - DETX (2):

The present invention generally relates to methods of controlling beta dimer formation during the production of recombinant hemoglobin. The invention is based on the discovery that the beta chain of human hemoglobin contains a histidine adjacent to the N-terminal amino acid that confers susceptibility to metal catalyzed **oxidation leading to the formation of stable** beta globin dimers.

Detailed Description Text - DETX (15):

Although beta dimerization can be useful for certain hemoglobin applications, it is not always desirable. Therefore, in one aspect, the present invention relates to methods of preventing the formation of such beta dimers. Such methods are accomplished by **mutations** in the beta globin. For example, in one embodiment, the histidine adjacent to the N-terminal amino acid in the beta globin can be substituted with one or more amino acids so that the expressed protein does not have a histidine in the second position from the N-terminus. Similarly, one or more amino acids can be inserted between the N-terminal amino acid and histidine as long as the inserted amino acid is not an amino acid directing N-terminal Met removal, for example, alanine, glycine, proline, serine, threonine, valine and **cysteine** if the beta globin in expressed by an E.coli host cell (Hirel et al., Proc. Nat'l Acad. Sci. U.S.A., 86:8247-51 (1989)). For example, in the E.coli system, the initating **methionine** (Met) residue is quantitatively removed by endogenous E.coli methionylaminopeptidase when the second amino acid expressed is alanine. Therefore, if alanine is inserted in front of the histidine, the resulting expressed protein would contain a histidine at the second position due to the cleavage of Met. Methods for the addition or substitution of amino acids can be accomplished by means known in the art or as described in the Examples below.

US-PAT-NO: 6159687

DOCUMENT-IDENTIFIER: US 6159687 A

TITLE: Methods for generating recombined polynucleotides

DATE-ISSUED: December 12, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vind; Jesper	Lyngby	N/A	N/A	DK

APPL-NO: 09/ 040697

DATE FILED: March 18, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. 119 of Danish applications 0307/97 filed Mar. 18, 1997, 0434/97 filed Apr. 17, 1997, 0625/97 filed May 30, 1997, and U.S. Provisional applications Ser. No. 60/044,836 filed Apr. 25, 1997 and 60/053,012 filed Jun. 24, 1997 the contents of which are fully incorporated herein by reference.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	0307/97	March 18, 1997
DK	0434/97	April 17, 1997
DK	0625/97	May 30, 1997

US-CL-CURRENT: 435/6, 435/91.2 , 435/91.5

ABSTRACT:

A method for in vitro construction of a library of recombined homologous polynucleotides from a number of different starting DNA templates and primers by induced template shifts during an polynucleotide synthesis is described, whereby

A. extended primers are synthesized by

a) denaturing the DNA templates

b) annealing primers to the templates,

c) extending the said primers by use of a polymerase,

d) stop the synthesis, and

e) separate the extended primers from the templates,

B. a template shift is induced by

a) isolating the extended primers from the templates and repeating steps A.b) to A.e) using the extended primers as both primers and templates, or

b) repeating steps A.b) to A.e),

C. this process is terminated after an appropriate number of cycles of process steps A. and B.a), A. and B.b), or combinations thereof.

Optionally the polynucleotides are amplified in a standard PCR reaction with specific primers to selectively amplify homologous polynucleotides of interest.

126 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (113):
Obviously the substitution of only one base within a codon doesn't provide total random **mutagenesis** (at protein level) as only a limited set of amino acid substitutions can be obtained by one base substitution at DNA level (e.g. **Methionine** encoded by ATG-codon requires three base substitution to become the TGT or TGC-codon encoding **Cysteine**).

Brief Summary Text - BSTX (128):
In the context of the present invention the term "positive polypeptide variants" means resulting polypeptide variants possessing functional properties which has been improved in comparison to the polypeptides producible from the corresponding input DNA sequences. Examples, of such improved properties can be as different as e.g. enhance or lowered biological activity, increased wash performance, thermostability, **oxidation stability,** substrate specificity, antibiotic resistance etc.

US-PAT-NO: 6136553

DOCUMENT-IDENTIFIER: US 6136553 A

TITLE: Mutant proteolytic enzymes and method of production

DATE-ISSUED: October 24, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Christianson; Teresa	Cotati	CA	94931	N/A
Goddette; Dean	Rohnert Park	CA	94928	N/A
Ladin; Beth Frances	Santa Rosa	CA	95405	N/A
Lau; Maria R.	Fairfield	CA	94533	N/A
Paech; Christian	Santa Rosa	CA	95403	N/A
Reynolds; Robert B.	Santa Rosa	CA	95404	N/A
Wilson; Charles R.	Santa Rosa	CA	95401	N/A
Yang; Shiow-Shong	Santa Rosa	CA	95401	N/A

APPL-NO: 08/ 980135

DATE FILED: November 26, 1997

PARENT-CASE:

This is a divisional application of copending application Ser. No. 08/618,446, filed Mar. 19, 1996, which is a divisional application of application Ser. No. 08/254,021, filed Jun. 2, 1994, now U.S. Pat. No. 5,500,364, which was a divisional application of application Ser. No. 07/706,691, filed May 29, 1991, now U.S. Pat. No. 5,340,735.

US-CL-CURRENT: 435/23, 435/219 , 435/220 , 435/221 , 435/24 , 702/19

ABSTRACT:

Mutant B. lentus DSM 5483 proteases are derived by the replacement of at least one amino acid residue of the mature form of the B. lentus DSM 5483 alkaline protease. The mutant proteases are expressed by genes which are mutated by site-specific mutagenesis. The amino acid sites selected for replacement are identified by means of a computer based method which compares the three dimensional structure of the wild-type protease and a reference protease.

23 Claims, 18 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

---------- KWIC ---------

Brief Summary Text - BSTX (5):

Subtilisins are a family of extracellular proteins having molecular weights in the range of 25,000-35,000 daltons and are produced by various Bacillus species. These proteins function as peptide hydrolases in that they catalyze the hydrolysis of peptide linkages in protein substrates at neutral and alkaline pH values. Subtilisins are termed serine proteases because they contain a specific serine residue which participates in the catalytic hydrolysis of peptide substrates. A subtilisin enzyme isolated from soil samples and produced by Bacillus lentus for use in detergent formulations having increased protease and **oxidative stability** over commercially available enzymes under conditions of pH 7 to 10 and at temperature of 10 to 60.degree. C. in aqueous solutions has been disclosed in copending patent application Ser. No. 07/398,854, filed on Aug. 25, 1989. This B. lentus alkaline protease enzyme (BLAP, vide infra) is obtained in commercial quantities by cultivating a Bacillus licheniformis ATCC 53926 strain which had been transformed by an expression plasmid which contained the wild type BLAP gene and the B. licheniformis ATCC 53926 alkaline protease gene promoter.

Brief Summary Text - BSTX (6):

Industrial processes generally are performed under physical conditions which require highly stable enzymes. Enzymes may be inactivated by high temperatures, pH extremes, oxidation, and surfactants. Even though Bacillus subtilisin proteases are currently used in many industrial applications, including detergent formulations, stability improvements are still needed. Market trends are toward more concentrated detergent powders, and an increase in liquid formulations. Increased shelf **stability and oxidative stability,** with retention of catalytic efficiency are needed. It is therefore desirable to isolate novel enzymes with increased stability, or to improve the stability of existing enzymes, including subtilisin proteases such as BLAP.

Brief Summary Text - BSTX (13):

EP 0251446 teaches the construction of mutant carbonyl hydrolases (proteases) which have at least one property different from the parental carbonyl hydrolase. It describes mutations which effect (either improve or decrease) **oxidative stability,** substrate specificity, catalytic activity, thermal stability, alkaline stability, pH activity profile, and resistance to autoproteolysis. These mutations were selected for introduction into Bacillus amyloliquefaciens subtilisin BPN' after alignment of the primary sequences of BPN' and proteases from B. subtilis, B. licheniformis, and thermitase. Such alignment can then be used to select amino acids in these other proteases which differ, as substitutes for the equivalent amino acid in the B. amyloliquefaciens carbonyl hydrolase. This application also describes alignment on the basis of a 1.8 .ANG. X-ray crystal structure of the B. amyloliquefaciens protease. Amino acids in the carbonyl hydrolase of B. amyloliquefaciens which when altered can affect stability, substrate specificity, or catalytic efficiency include: Met50, Met124, and Met222 for **oxidative stability**; Tyr104, Ala152, Glu156, Gly166, Gly169, Phe189, and Tyr217 for substrate specificity; N155 alterations were found to decrease turnover,

and lower Km; Asp36, Ile107, Lys170, Asp197, Ser204, Lys213, and Met222 for alkaline stability; and Met199, and Tyr21 for thermal stability. Alteration of other amino acids was found to affect multiple properties of the protease. Included in this category are Ser24, Met50, Asp156, Gly166, Gly169, and Tyr217. Substitution at residues Ser24, Met50, Ile107, Glu156, Gly166, Gly169, Ser204, Lys213, Gly215, and Tyr217 was predicted to increase thermal and alkaline stability. An important point about this patent application is that with the exception of those mutations effecting substrate specificity, no rational mutational approach for improving the alkaline or temperature stability of a protease based upon computer simulations of an X-ray crystal structure is described.

Brief Summary Text - BSTX (17):

Sensitivity to oxidation is an important deficiency of serine proteases used in detergent applications (Stauffer, C. E., and Etson, D. (1969) J. Biol. Chem. 244:5333-5338). EP 0130756, EP 0247647, and U.S. Pat. No. 4,760,025 teach a saturation **mutation** method where one or multiple **mutations** are introduced into the subtilisin BPN' at amino acid residues Asp32, Asn155, Tyr104, Met222, Gly166, His64, Ser221, Gly169, Glu156, Ser33, Phe189, Tyr217, and/or Ala152. Using this approach **mutant** proteases exhibiting improved **oxidative stability,** altered substrate specificity, and/or altered pH activity profiles are obtained. A method is taught in which improved **oxidative stability** is achieved by substitution of **methionine, cysteine,** tryptophan, and lysine residues. These publications also teach that **mutations** within the active site region of the protease are also most likely to influence activity. Random or selected **mutations** can be introduced into a target gene using the experimental approach but neither EP 0130756, EP 0247647, nor U.S. Pat. No. 4,760,025 teach a method for predicting amino acid alterations which will improve the thermal or surfactant stability of the protease.

US-PAT-NO: 6080568

DOCUMENT-IDENTIFIER: US 6080568 A

TITLE: Mutant .alpha.-amylase comprising modification at residues corresponding to A210, H405 and/or T412 in Bacillus licheniformis

DATE-ISSUED: June 27, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Day; Anthony G.	San Francisco	CA	N/A	N/A
Swanson; Barbara A.	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 914679

DATE FILED: August 19, 1997

US-CL-CURRENT: 435/202, 435/201 , 435/203 , 435/275 , 435/440 , 435/832 , 435/836 , 510/320 , 570/226 , 570/235

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which one or more of residues corresponding to A210, H405 and T412 in Bacillus licheniformis are mutated. The disclosed .alpha.-amylase enzymes show altered or improved stability and/or activity profiles.

11 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

---------- KWIC --------

Brief Summary Text - BSTX (16):

In PCT Publication No. WO 95/35382, a mutant .alpha.-amylase is described having improved **oxidation stability** and having changes at positions 104, 128, 187 and/or 188 in B. licheniformis .alpha.-amylase.

Brief Summary Text - BSTX (18):

In PCT Publication No. WO 94/02597, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more methionines are replaced by any amino acid except **cysteine r methionine**.

Brief Summary Text - BSTX (19):

In PCT publication No. WO 94/18314, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more of the **methionine,** tryptophan, **cysteine,** histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Detailed Description Text - DETX (13):

.alpha.-Amylases according to the present invention which exhibit altered performance characteristics providing desirable and unexpected results are useful in the various applications for which .alpha.-amylases are commonly used. For example, .alpha.-amylases according to the present invention which exhibit altered performance characteristics at low pH, including improved thermostability, improved pH **stability and/or improved oxidative stability,** are useful in low pH liquefaction of starch. Enhanced thermostability will be useful in extending the shelf life of products which incorporate them. Enhanced **oxidative stability** or improved performance is particularly desirable in cleaning products, and for extending the shelf life of .alpha.-amylase in the presence of bleach, perborate, percarbonate or peracids used in such cleaning products. To the contrary, reduced thermal **stability or oxidative stability** may be useful in industrial processes which require the rapid and efficient quenching of amylolytic activity.

Detailed Description Text - DETX (14):

.alpha.-Amylases of the present invention which exhibit improved low pH stability will be especially useful in starch processing and particularly in starch liquefaction. Conditions present during commercially desirable liquefaction processes characteristically include low pH, high temperature and potential oxidation conditions requiring .alpha.-amylases exhibiting improved low pH performance, improved thermal **stability and improved oxidative stability**. Accordingly, .alpha.-amylases according to the present invention which are particularly useful in liquefaction exhibit improved performance at a pH of less than about 6, preferably less than about 5.5, and most preferably less than about 5.0. Additionally, .alpha.-amylases according to the present invention which exhibit increased thermal stability at temperatures of between about 80-120.degree. C., and preferably between about 100-110.degree. C., and increased stability in the presence of oxidants will be particularly useful.

Detailed Description Text - DETX (17):

In another embodiment of the present invention, detergent compositions in either liquid, gel or granular form, which comprise the .alpha.-amylase according to the present invention may be useful. Such detergent compositions will particularly benefit from the addition of an .alpha.-amylase according to the present invention which has increased thermal stability to improve shelf-life or increased **oxidative stability** such that the .alpha.-amylase has improved resistance to bleach or peracid compounds commonly present in detergents. Thus, .alpha.-amylase according to the present invention may be advantageously formulated into known powdered, liquid or gel detergents having a pH of between about 6.5 and about 12.0. Detergent compositions comprising

the .alpha.-amylase according to the present invention may further include other enzymes such as endoglycosidases, cellulases, proteases, lipases or other amylase enzymes, particularly .alpha.-amylase derived from Bacillus stearothermophilus, as well as additional ingredients as generally known in the art.

Detailed Description Text - DETX (20):

Embodiments of the present invention which comprise a combination of the .alpha.-amylase according to the present invention with protease enzymes preferably include **oxidatively stable** proteases such as those described in U.S. Re. 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease mutants (**oxidatively stable** proteases), and particularly such mutants having a substitution for the methionine at a position equivalent to M222 in Bacillus amyloliquefaciens, are described in U.S. Re. 34,606.

Detailed Description Text - DETX (23):

The improved .alpha.-amylases according to the present invention are contemplated to provide important advantages when compared to wild type Bacillus .alpha.-amylases. For example, one advantage is the increased activity found at low pH and high temperatures typical of common starch liquefaction methods. Other advantages may include increased high pH and **oxidative stability** which facilitates their use in detergents; more complete hydrolysis of starch molecules is achieved which reduces residual starch in the processing stream; improved stability in the absence of calcium ion; and that the addition of equal protein doses of .alpha.-amylase according to the invention may provide superior performance when compared to wild type Bacillus licheniformis .alpha.-amylase due to improvements in both specific activity and stability under stressed conditions.

US-PAT-NO: 6008026

DOCUMENT-IDENTIFIER: US 6008026 A

TITLE: Mutant .alpha.-amylase having introduced therein a disulfide bond

DATE-ISSUED: December 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Day; Anthony G.	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 890383

DATE FILED: July 11, 1997

US-CL-CURRENT: 435/96, 435/202 , 435/203 , 435/204 , 435/205 , 435/262 , 435/267 , 435/274 , 435/275 , 435/320.1 , 536/23.2

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which one or more disulfide bonds are introduced into the enzyme via addition or substitution of a residue with a cysteine. The disclosed .alpha.-amylase enzymes show altered or improved stability and/or activity profiles.

15 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

---------- KWIC ---------

Brief Summary Text - BSTX (16):

In PCT Publication No. WO 95/35382, a mutant .alpha.-amylase is described having improved **oxidation stability** and having changes at positions 104, 128, 187 and/or 188 in B. licheniformis .alpha.-amylase.

Brief Summary Text - BSTX (18):

In PCT Publication No. WO 94/02597, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more methionines are replaced by any amino acid except **cysteine or methionine**.

Brief Summary Text - BSTX (19):

In PCT publication No. WO 94/18314, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more of the **methi nine,** tryptophan, **cysteine,** histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Detailed Description Text - DETX (15):

.alpha.-Amylases according to the present invention which exhibit altered performance characteristics providing desirable and unexpected results are useful in the various applications for which .alpha.-amylases are commonly used. For example, .alpha.-amylases according to the present invention which exhibit altered performance characteristics at low pH, including improved thermostability, improved pH **stability and/or improved oxidative stability,** are useful in low pH liquefaction of starch. Enhanced thermostability will be useful in extending the shelf life of products which incorporate them. Enhanced **oxidative stability** or improved performance is particularly desirable in cleaning products, and for extending the shelf life of .alpha.-amylase in the presence of bleach, perborate, percarbonate or peracids used in such cleaning products. To the contrary, reduced thermal **stability or oxidative stability** may be useful in industrial processes which require the rapid and efficient quenching of amylolytic activity.

Detailed Description Text - DETX (16):

.alpha.-Amylases of the present invention which exhibit improved low pH stability will be especially useful in starch processing and particularly in starch liquefaction. Conditions present during commercially desirable liquefaction processes characteristically include low pH, high temperature and potential oxidation conditions requiring .alpha.-amylases exhibiting improved low pH performance, improved thermal **stability and improved oxidative stability**. Accordingly, .alpha.-amylases according to the present invention which are particularly useful in liquefaction exhibit improved performance at a pH of less than about 6, preferably less than about 5.5, and most preferably less than about 5.0. Additionally, .alpha.-amylases according to the present invention which exhibit increased thermal stability at temperatures of between about 80-120.degree. C., and preferably between about 100-110.degree. C., and increased stability in the presence of oxidants will be particularly useful.

Detailed Description Text - DETX (19):

In another embodiment of the present invention, detergent compositions in either liquid, gel or granular form, which comprise the .alpha.-amylase according to the present invention may be useful. Such detergent compositions will particularly benefit from the addition of an .alpha.-amylase according to the present invention which has increased thermal stability to improve shelf-life or increased **oxidative stability** such that the .alpha.-amylase has improved resistance to bleach or peracid compounds commonly present in detergents. Thus, .alpha.-amylase according to the present invention may be advantageously formulated into known powdered, liquid or gel detergents having a pH of between about 6.5 and about 12.0. Detergent compositions comprising the .alpha.-amylase according to the present invention may further include other enzymes such as endoglycosidases, cellulases, proteases, lipases or other

amylase enzymes, particularly .alpha.-amylase derived from Bacillus stearothermophilus, as well as additional ingredients as generally known in the art.

Detailed Description Text - DETX (21):

Embodiments of the present invention which comprise a combination of the .alpha.-amylase according to the present invention with protease enzymes preferably include **oxidatively stable** proteases such as those described in U.S. Re. 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease mutants (**oxidatively stable** proteases), and particularly such mutants having a substitution for the methionine at a position equivalent to M222 in Bacillus amyloliquefaciens, are described in U.S. Re. 34,606.

Detailed Description Text - DETX (24):

The improved .alpha.-amylases according to the present invention are contemplated to provide several important advantages when compared to wild type Bacillus .alpha.-amylases. For example, one advantage is the increased activity found at low pH and high temperatures typical of common starch liquefaction methods. Another advantage is the increased high pH and **oxidative stability** which facilitates their use in detergents. Another advantage is that a more complete hydrolysis of starch molecules is achieved which reduces residual starch in the processing stream. Yet another advantage is their improved stability in the absence of calcium ion. Yet another advantage is that the addition of equal protein doses of .alpha.-amylase according to the invention provide superior performance when compared to wild type Bacillus licheniformis .alpha.-amylase due to improvements in both specific activity and stability under stressed conditions. In other words, because of the generally increased stability of the amylases according to the present invention, the increased specific activity on starch of the inventive amylases translates to even greater potential performance benefits of this variant. Under conditions where the wild type enzyme is being inactivated, not only does more of the inventive amylase survive because of its increased stability, but also that which does survive expresses proportionally more activity because of its increased specific activity.

Detailed Description Text - DETX (39):

A pBLapr plasmid having threonine substituted for **methionine** at amino acid 15 was constructed according to U.S. patent application Ser. No. 08/194,664 (PCT Publication No. WO 94/18314). To introduce the additional **cysteine** residues, the following **mutagenic** primers encoding for substitutions of E119C/S130C and D124C/R127C were used together with non-**mutagenic** primers to introduce the desired **mutations** into linear multiple tandem repeats of the plasmid by the method of multimerization as described below.

US-PAT-NO: 5985639

DOCUMENT-IDENTIFIER: US 5985639 A

TITLE: Mutant proteolytic enzymes and method of production

DATE-ISSUED: November 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Christianson; Teresa	Cotati	CA	N/A	N/A
Goddette; Dean	Rohnert Park	CA	N/A	N/A
Ladin; Beth Frances	Santa Rosa	CA	N/A	N/A
Lau; Maria R.	Fairfield	CA	N/A	N/A
Paech; Christian	Santa Rosa	CA	N/A	N/A
Reynolds; Robert B.	Santa Rosa	CA	N/A	N/A
Wilson; Charles R.	Santa Rosa	CA	N/A	N/A
Yang; Shiow-Shong	Santa Rosa	CA	N/A	N/A

APPL-NO: 08/ 618446

DATE FILED: March 19, 1996

PARENT-CASE:

This application is a divisional of application Ser. No. 08/254,021 filed Jun. 2, 1994 which issued as U.S. Pat. No. 5,500,364, which is a divisional of application Ser. No. 07/706,691 filed May 29, 1991 which issued as U.S. Pat. No. 5,340,735.

US-CL-CURRENT: 435/221, 435/220 , 435/252.31 , 435/320.1 , 435/471 , 435/69.1 , 435/69.7 , 536/23.2 , 536/23.5

ABSTRACT:

Mutant B. lentus DSM 5483 proteases are derived by the replacement of at least one amino acid residue of the mature form of the B. lentus DSM 5483 alkaline protease. The mutant proteases are expressed by genes which are mutated by site-specific mutagenesis. The amino acid sites selected for replacement are identified by means of a computer based method which compares the three dimensional structure of the wild-type protease and a reference protease.

48 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

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Brief Summary Text - BSTX (5):

Subtilisins are a family of extracellular proteins having molecular weights in the range of 25,000-35,000 daltons and are produced by various Bacillus species. These proteins function as peptide hydrolases in that they catalyze the hydrolysis of peptide linkages in protein substrates at neutral and alkaline pH values. Subtilisins are termed serine proteases because they contain a specific serine residue which participates in the catalytic hydrolysis of peptide substrates. A subtilisin enzyme isolated from soil samples and produced by Bacillus lentus for use in detergent formulations having increased protease and **oxidative stability** over commercially available enzymes under conditions of pH 7 to 10 and at temperature of 10 to 60.degree. C. in aqueous solutions has been disclosed in copending patent application Ser. No. 07/398,854, filed on Aug. 25, 1989. This B. lentus alkaline protease enzyme (BLAP, vide infra) is obtained in commercial quantities by cultivating a Bacillus licheniformis ATCC 53926 strain which had been transformed by an expression plasmid which contained the wild type BLAP gene and the B. licheniformis ATCC 53926 alkaline protease gene promoter.

Brief Summary Text - BSTX (6):

Industrial processes generally are performed under physical conditions which require highly stable enzymes. Enzymes may be inactivated by high temperatures, pH extremes, oxidation, and surfactants. Even though Bacillus subtilisin proteases are currently used in many industrial applications, including detergent formulations, stability improvements are still needed. Market trends are toward more concentrated detergent powders, and an increase in liquid formulations. Increased shelf **stability and oxidative stability,** with retention of catalytic efficiency are needed. It is therefore desirable to isolate novel enzymes with increased stability, or to improve the stability of existing enzymes, including subtilisin proteases such as BLAP.

Brief Summary Text - BSTX (13):

EP 0251446 teaches the construction of mutant carbonyl hydrolases (proteases) which have at least one property different from the parental carbonyl hydrolase. It describes mutations which effect (either improve or decrease) **oxidative stability,** substrate specificity, catalytic activity, thermal stability, alkaline stability, pH activity profile, and resistance to autoproteolysis. These mutations were selected for introduction into Bacillus amyloliquefaciens subtilisin BPN' after alignment of the primary sequences of BPN' and proteases from B. subtilis, B. licheniformis, and thermitase. Such alignment can then be used to select amino acids in these other proteases which differ, as substitutes for the equivalent amino acid in the B. amyloliquefaciens carbonyl hydrolase. This application also describes alignment on the basis of a 1.8 .ANG. X-ray crystal structure of the B. amyloliquefaciens protease. Amino acids in the carbonyl hydrolase of B. amyloliquefaciens which when altered can affect stability, substrate specificity, or catalytic efficiency include: Met50, Met124, and Met222 for **oxidative stability**; Tyr104, Ala152, Glu156, Gly166, Gly169, Phe189, and Tyr217 for substrate specificity; N155 alterations were found to decrease turnover,

and lower Km; Asp36, Ile107, Lys170, Asp197, Ser204, Lys213, and Met222 for alkaline stability; and Met199, and Tyr21 for thermal stability. Alteration of other amino acids was found to affect multiple properties of the protease. Included in this category are Ser24, Met50, Asp156, Gly166, Gly169, and Tyr217. Substitution at residues Ser24, Met50, Ile107, Glu156, Gly166, Gly169, Ser204, Lys213, Gly215, and Tyr217 was predicted to increase thermal and alkaline stability. An important point about this patent application is that with the exception of those mutations effecting substrate specificity, no rational mutational approach for improving the alkaline or temperature stability of a protease based upon computer simulations of an X-ray crystal structure is described.

Brief Summary Text - BSTX (17):

Sensitivity to oxidation is an important deficiency of serine proteases used in detergent applications (Stauffer, C. E., and Etson, D. (1969) J. Biol. Chem. 244:5333-5338). EP 0130756, EP 0247647, and U.S. Pat. No. 4,760,025 teach a saturation **mutation** method where one or multiple **mutations** are introduced into the subtilisin BPN' at amino acid residues Asp32, Asn155, Tyr104, Met222, Gly166, His64, Ser221, Gly169, Glu156, Ser33, Phe189, Tyr217, and/or Ala152. Using this approach **mutant** proteases exhibiting improved **oxidative stability,** altered substrate specificity, and/or altered pH activity profiles are obtained. A method is taught in which improved **oxidative stability** is achieved by substitution of **methionine, cysteine,** tryptophan, and lysine residues. These publications also teach that **mutations** within the active site region of the protease are also most likely to influence activity. Random or selected **mutations** can be introduced into a target gene using the experimental approach but neither EP 0130756, EP 0247647, nor U.S. Pat. No. 4,760,025 teach a method for predicting amino acid alterations which will improve the thermal or surfactant stability of the protease.

US-PAT-NO: 5958739

DOCUMENT-IDENTIFIER: US 5958739 A

TITLE: Mutant .alpha.-amylase

DATE-ISSUED: September 28, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitchinson; Colin	Palo Alto	CA	N/A	N/A
Requadt; Carol	Palo Alto	CA	N/A	N/A
Ropp; Traci	Palo Alto	CA	N/A	N/A
Solheim; Leif P.	Palo Alto	CA	N/A	N/A
Ringer; Christopher	Palo Alto	CA	N/A	N/A
Day; Anthony	Palo Alto	CA	N/A	N/A

APPL-NO: 08/ 704706

DATE FILED: February 20, 1997

PCT-DATA:
APPL-NO: PCT/US96/09089
DATE-FILED: June 6, 1996
PUB-NO: WO96/39528
PUB-DATE: Dec 19, 1996
371-DATE: Feb 20, 1997
102(E)-DATE:Feb 20, 1997

US-CL-CURRENT: 435/99, 435/201 , 435/202 , 435/203 , 435/204 , 435/252.3 , 435/252.31 , 435/254.11 , 435/320.1 , 435/325 , 435/410 , 510/226 , 510/300 , 510/305 , 510/320 , 510/374 , 510/392 , 536/23.2

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which one or more asparagine residues are substituted with a different amino acid or deleted. The disclosed .alpha.-amylase enzymes show altered or improved low pH starch hydrolysis performance, stability and activity profiles.

32 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

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Brief Summary Text - BSTX (16):
In PCT Publication No. WO 94/02597, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more methionines are replaced by any amino acid except **cysteine or methionine**.

Brief Summary Text - BSTX (17):
In PCT publication No. WO 94/18314, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more of the **methionine,** tryptophan, **cysteine,** histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Brief Summary Text - BSTX (20):
Despite the advances made in the prior art, a need exists for an .alpha.-amylase which is effective enough at low pH values to allow commercial liquefaction at lower pH than currently practical. Similarly, a need exists in the art for a method which allows efficient liquefaction of dry milled grain at high temperatures. Further, a need exists in the art for a method which allows the efficient liquefaction of starch with a decreased reliance on the costly addition of calcium. Additionally, a need exists for a more efficient enzyme to effect a more complete hydrolysis of starch at the liquefaction stage to ensure efficient saccharification. Because commercially available amylases are not acceptable under many conditions due to stability problems, for example, the high alkalinity and oxidant (bleach) levels associated with detergents, there is a need for an amylase having altered, and preferably increased, performance profiles under such conditions. Thus, altered performance characteristics such as increased activity, thermostability, pH **stability, oxidative stability** or calcium stability which can be achieved while also altering, maintaining, or increasing enzymatic activity as compared to the wild type or precursor enzyme, would be desirable.

Brief Summary Text - BSTX (22):
It is an object of the present invention to provide an .alpha.-amylase having altered performance profiles, such as pH **stability, alkaline stability, oxidative stability** or enzymatic activity.

Detailed Description Text - DETX (14):
The .alpha.-amylases according to the present invention exhibit altered performance characteristics providing desirable and unexpected results which are useful in the various applications for which .alpha.-amylases are commonly used. For example, .alpha.-amylases according to the present invention which exhibit altered performance characteristics at low pH, including improved thermostability, improved pH **stability and/or improved oxidative stability,** are useful in low pH liquefaction of starch. Enhanced thermostability will be useful in extending the shelf life of products which incorporate them. Enhanced **oxidative stability** or improved performance is particularly desirable in cleaning products, and for extending the shelf life of .alpha.-amylase in the presence of bleach, perborate, percarbonate or peracids used in such

cleaning products. To the contrary, reduced thermal **stability or xidative stability** may be useful in industrial processes which require the rapid and efficient quenching of amylolytic activity.

Detailed Description Text - DETX (15):

The .alpha.-amylase of the present invention is especially useful in starch processing and particularly in starch liquefaction. Conditions present during commercially desirable liquefaction processes characteristically include low pH, high temperature and potential oxidation conditions requiring .alpha.-amylases exhibiting improved low pH performance, improved thermal **stability and improved oxidative stability**. Accordingly, .alpha.-amylases according to the present invention which are particularly useful in liquefaction exhibit improved performance at a pH of less than about 6, preferably less than about 5.5, and most preferably between about 5.0 and 5.5. Additionally, .alpha.-amylases according to the present invention which exhibit increased thermal stability at temperatures of between about 80-120.degree. C., and preferably between about 100-110.degree. C., and increased stability in the presence of oxidants will be particularly useful. Preferably, the .alpha.-amylase according to the present invention which is used in liquefaction, in addition to deletion or substitution of an asparagine, further comprises a deletion or substitution at one or more residues corresponding to M15, V128, H133, W138, S187, M197 and/or A209 in Bacillus licheniformis. In a more preferred embodiment, .alpha.-amylase used in starch liquefaction according to the present invention comprises a deletion or substitution corresponding to position N188. Most preferably, the amylase comprises a substitution corresponding to M15T/N188S, M15L/N188S, M15T/H133Y/N188S, M15T/H133Y/N188S/A209V, M15T/N188S/A209V, M15T/V128E/H133Y/N188S, M15T/S187D/N188S, M15T/H13 or M15T/H 133Y/A209V in Bacillus licheniformis.

Detailed Description Text - DETX (18):

In another embodiment of the present invention there are provided detergent compositions in either liquid, gel or granular form, which comprise the .alpha.-amylase according to the present invention. Such detergent compositions will particularly benefit from the addition of an .alpha.-amylase according to the present invention which has increased thermal stability to improve shelf-life or increased **oxidative stability** such that the .alpha.-amylase has improved resistance to bleach or peracid compounds commonly present in detergents. Thus, .alpha.-amylase according to the present invention may be advantageously formulated into known powdered, liquid or gel detergents having a pH of between about 6.5 and about 12.0. A preferred embodiment of the present invention further comprises the deletion or substitution of a methionine residue or a tryptophan residue, for example M15, M197 or W138 as described in commonly assigned U.S. patent application Ser. Nos. 08/289,351 and 08/409,771, the disclosures of which are incorporated by reference; substitution at M133Y as described in PCT Publication No. WO 91/00353; or substitution at A209 as described in DeClerck, et al., J. Biol. Chem., vol. 265, pp. 15481-15488 (1990). Also preferably, an .alpha.-amylase according to the present invention used in detergent compositions comprises a deletion or substitution at position N188. Detergent compositions comprising the .alpha.-amylase according to the present invention may further include other enzymes such as endoglycosidases, cellulases, proteases, lipases or other

amylase enzymes, particularly .alpha.-amylase derived from Bacillus stearothermophilus, as well as additional ingredients as generally known in the art.

Detailed Description Text - DETX (19):

Embodiments of the present invention which comprise a combination of the .alpha.-amylase according to the present invention with protease enzymes preferably include **oxidatively stable** proteases such as those described in U.S. Re. Pat. No. 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk), MAXAPEM (Gist-brocades) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease mutants (**oxidatively stable** proteases), and particularly such mutants having a substitution for the methionine at a position equivalent to M222 in Bacillus amyloliquefaciens, are described in U.S. Re. Pat. No. 34,606.

Detailed Description Text - DETX (22):

The improved .alpha.-amylases according to the present invention provide several important advantages when compared to wild type Bacillus .alpha.-amylases. For example, one advantage is the increased activity found at low pH and high temperatures typical of common starch liquefaction methods. Another advantage is the increased high pH and **oxidative stability** which facilitates their use in detergents. Another advantage is that a more complete hydrolysis of starch molecules is achieved which reduces residual starch in the processing stream. Yet another advantage is their improved stability in the absence of calcium ion. Yet another advantage is that the addition of equal protein doses of .alpha.-amylase according to the invention provide superior performance when compared to wild type Bacillus licheniformis .alpha.-amylase due to improvements in both specific activity and stability under stressed conditions. In other words, because of the generally increased stability of the amylases according to the present invention, the increased specific activity on starch of the inventive amylases translates to even greater potential performance benefits of this variant. Under conditions where the wild type enzyme is being inactivated, not only does more of the inventive amylase survive because of its increased stability, but also that which does survive expresses proportionally more activity because of its increased specific activity.

US-PAT-NO: 5849549

DOCUMENT-IDENTIFIER: US 5849549 A

TITLE: **Oxidatively stable** alpha-amylase

DATE-ISSUED: December 15, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barnett; Christopher C.	South San Franciso	CA	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Power; Scott D.	San Bruno	CA	N/A	N/A
Requadt; Carol A.	Tiburon	CA	N/A	N/A

APPL-NO: 08/ 468698

DATE FILED: June 6, 1995

PARENT-CASE:

RELATED APPLICATION

This is a divisional of U.S. Ser. No. 08/194,664 filed Feb. 10, 1994, now pending, which is a continuation-in-part of U.S. Ser. No. 08/016,395 filed Feb. 11, 1993, abandoned.

US-CL-CURRENT: 435/99, 435/202 , 536/23.2

ABSTRACT:

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such mutant alpha-amylases have altered **oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

2 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

---------- KWIC ---------

Abstract Text - ABTX (1):

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such mutant alpha-amylases have altered **oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

TITLE - TI (1):

Oxidatively stable alpha-amylase

Brief Summary Text - BSTX (2):

The present invention relates to novel alpha-amylase mutants having an amino acid sequence not found in nature, such mutants having an amino acid sequence wherein one or more amino acid residue(s) of a precursor alpha-amylase, specifically any oxidizable amino acid, have been substituted with a different amino acid. The mutant enzymes of the present invention exhibit altered stability/activity profiles including but not limited to altered **oxidative stability,** altered pH performance profile, altered specific activity and/or altered thermostability.

Brief Summary Text - BSTX (7):

In order to alter the stability or activity profile of amylase enzymes under varying conditions, it has been found that selective replacement, substitution or deletion of oxidizable amino acids, such as a **methionine,** tryptophan, tyrosine, histidine or **cysteine,** results in an altered profile of the variant enzyme as compared to its precursor. Because currently commercially available amylases are not acceptable (stable) under various conditions, there is a need for an amylase having an altered stability andlor activity profile. This altered **stability (oxidative,** thermal or pH performance profile) can be achieved while maintaining adequate enzymatic activity, as compared to the wild-type or precursor enzyme. The characteristic affected by introducing such **mutations** may be a change in **oxidative stability** while maintaining thermal stability or vice versa. Additionally, the substitution of different amino acids for an oxidizable amino acids in the alpha-amylase precursor sequence or the deletion of one or more oxidizable amino acid(s) may result in altered enzymatic activity at a pH other than that which is considered optimal for the precursor alpha-amylase. In other words, the **mutant** enzymes of the present invention may also have altered pH performance profiles, which may be due to the enhanced **oxidative stability** of the enzyme.

Brief Summary Text - BSTX (10):

Preferably the substitution or deletion of one or more amino acids in the amino acid sequence is due to the replacement or deletion of one or more methionine, tryptophan, cysteine, histidine or tyrosine residues in such sequence, most preferably the residue which is changed is a methionine residue. The oxidizable amino acid residues may be replaced by any of the other 20 naturally occurring amino acids. If the desired effect is to alter the **oxidative stability** of the precursor, the amino acid residue may be substituted with a non-oxidizable amino acid (such as alanine, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, isoleucine, leucine, tysine, phenylalanine, proline, serine, threonine, or valine) or another oxidizable amino acid (such as cysteine, methionine, tryptophan, tyrosine or histidine, listed in order of most easily oxidizable to less readily oxidizable). Likewise, if the desired effect is to alter thernostability, any of the other 20 naturally occurring amino acids may be substituted (i.e., cysteine may be substituted for methionine).

Brief Summary Text - BSTX (11):

Preferred **mutants** comprise the substitution of a **methionine** residue equivalent to any of the **methionine** residues found in B. licheniformis alpha-amylase (+8, +15, +197, +256, +304, +366 and +438). Most preferably the **methionine** to be replaced is a **methionine** at a position equivalent to position +197 or +15 in B. licheniforrnis alpha-amylase. Preferred substitute amino acids to replace the **methionine** at position +197 are alanine (A), isoleucine (I), threonine (T) or **cysteine** (C). The preferred substitute amino acids at position +15 are leucine (L), threonine (T), asparagine (N), aspartate (D), serine (S), valine (V) and isoleucine (I), although other substitute amino acids not specified above may be useful. Two specifically preferred **mutants** of the present invention are M197T and M15L.

Brief Summary Text - BSTX (13):

The alpha-amylase mutants of the present invention, in general, exhibit altered **oxidative stability** in the presence of hydrogen peroxide and other oxidants such as bleach or peracids, or, more specific, milder oxidants such as chloramine-T. Mutant enzymes having enhanced **oxidative stability** will be useful in extending the shelf life and bleach, perborate, percarbonate or peracid compatibility of amylases used in cleaning products. Similarly, reduced **oxidative stability** may be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity. The mutant enzymes of the present invention may also demonstrate a broadened pH performance profile whereby mutants such as M15L show stability for low pH starch liquefaction and mutants such as M197T show stability at high pH cleaning product conditions. The mutants of the present invention may also have altered thermal stability whereby the mutant may have enhanced stability at either high or low temperatures. It is understood that any change (increase or decrease) in the mutant's enzymatic characteristic(s), as compared to its precursor, may be beneficial depending on the desired end use of the mutant alpha-amylase.

Detailed Description Text - DETX (2):

It is believed that amylases used in starch liquefaction may be subject to some form of inactivation due to some activity present in the starch slurry (see commonly owned U.S. application Ser. Nos. 07/785,624 and 071785,623 and U.S. Pat. No. 5,180,669, issued Jan. 19, 1993, incorporated herein by reference). Furthermore, use of an amylase in the presence of oxidants, such as in bleach or peracid containing detergents, may result in partial or complete inactivation of the amylase. Therefore, the present invention focuses on altering the oxidative sensitivity of amylases. The mutant enzymes of the present invention may also have an altered pH profile and/or altered thermal **stability which may be due to the enhanced oxidative stability** of the enzyme at low or high pH's.

Detailed Description Text - DETX (7):

Specific residues corresponding to positions M197, M15 and W138 of Bacillus licheniformis alpha-amylase are identified herein for substitution or deletion, as are all **methionine,** histidine, tryptophan, **cysteine** and tymsine positions. The amino acid position number (i.e., +197) refers to the number assigned to the mature Bacillus licheniformis alpha-amylase sequence presented in FIG. 2. The invention, however, is not limited to the **mutation** of this particular mature alpha-amylase (B. licheniformis) but extends to precursor alpha-amylases containing amino acid residues at positions which are equivalent to the particular identified residue in.B. licheniformis alpha-amylase residue (amino acid) of a precursor alpha-amylase is equivalent to a residue of B. licheniformis alpha-amylase if it is either homologous (i.e., corresponding in position in either primary or tertiary structure) or analogous to a specific residue or portion of that residue in B. licheniformis alpha-amylase (i.e., having the same or similar functional capacity to combine, react, or interact chemically or structurally).

Detailed Description Text - DETX (14):

Based on the conditions of a preferred liquefaction process, as described in commonly owned U.S. application Ser. Nos. 071788,624 and 071785,623 and U.S. Pat. No. 5,180,669, namely low pH, high temperature and potential oxidation conditions, preferred mutants of the present invention for use in liquefaction processes comprise mutants exhibiting altered pH performance profiles (i.e., low pH profile, pH <6 and preferably pH <5.5), and/or altered thermal stability (i.e., high temperature, about 90.degree.-110.degree. C.), and/or altered **oxidative stability (i.e., enhanced oxidative stability)**.

Detailed Description Text - DETX (66):

As can be seen in FIG. 9, there was a wide range of amylase activity (measured in the soluble substrate assay) expressed by the M197X (A4 form) variants. The amylases were partially purified from the supematants by precipitation with two volumes of ethanol and resuspension. They were then screened for thermal stability (FIG. 10) by heating at 95.degree. C. for 5 minutes in 10 mM acetate buffer pH 5.0, in the presence of 5 mM calcium chloride; the A4 wild-type retained 28% of its activity after incubation. For M197W and M197P we were unable to recover active protein from the supernatants.

Upon sequencing, the M197H variant was found to contain a second mutation, N190K. M197L was examined in a separate experiment and was one of the lowest thermally stable variants. There appears to be a broad correlation between expression of amylase activity and thermal stability. The licheniformnis amylase is restricted in what residues it can accommodate at position 197 in terms of retaining or enhancing thermal stability: cysteine and threonine are preferred for maximal thermal stability under these conditions whereas alanine and isoleucine are of intermediate stability. However, other substitutions at position +197 result in lowered thermal stability which may be useful for other applications. Additionally, different substitutions at +197 may have other beneficial properties, such as altered pH performance profile or altered **oxidative stability**. For example, the M197C variant was found to inactivate readily by air **oxidation but had enhanced thermal stability**. Conversely, compared to the M197L variant, both M197T and M197A retained not only high thermal stability (FIG. 10), but also high activity (Table III), while maintaining resistance to inactivation by peroxide at pH 5 to pH 10 (FIG. 7).

US-PAT-NO: 5824532

DOCUMENT-IDENTIFIER: US 5824532 A

TITLE: **Oxidativley stable** alpha-amylase

DATE-ISSUED: October 20, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barnett; Christopher C.	South San Francisco	CA	N/A	N/A
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Power; Scott D.	San Bruno	CA	N/A	N/A
Requadt; Carol A.	Tiburon	CA	N/A	N/A

APPL-NO: 08/ 468220

DATE FILED: June 6, 1995

PARENT-CASE:

RELATED APPLICATIONS

This is a divisional of U.S. Ser. No. 08/194,664 filed Feb. 10, 1994, now pending which is a continuation-in-part of U.S. Ser. No. 08/016,395 filed Feb. 11, 1993 now abandoned.

US-CL-CURRENT: 435/202, 435/201 , 435/203 , 435/204 , 435/252.3 , 435/252.31 , 435/320.1 , 435/71.2 , 536/23.2 , 536/23.7

ABSTRACT:

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such mutant alpha-amylases have altered **oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

11 Claims, 28 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 22

---------- KWIC ---------

Abstract Text - ABTX (1):

Novel alpha-amylase mutants derived from the DNA sequences of naturally occurring or recombinant alpha-amylases are disclosed. The mutant alpha-amylases, in general, are obtained by in vitro modifications of a precursor DNA sequence encoding the naturally occurring or recombinant alpha-amylase to generate the substitution (replacement) or deletion of one or more oxidizable amino acid residues in the amino acid sequence of a precursor alpha-amylase. Such mutant alpha-amylases have altered **oxidative stability** and/or altered pH performance profiles and/or altered thermal stability as compared to the precursor. Also disclosed are detergent and starch liquefaction compositions comprising the mutant amylases, as well as methods of using the mutant amylases.

TITLE - TI (1):

Oxidativley stable alpha-amylase

Brief Summary Text - BSTX (2):

The present invention relates to novel alpha-amylase mutants having an amino acid sequence not found in nature, such mutants having an amino acid sequence wherein one or more amino acid residue(s) of a precursor alpha-amylase, specifically any oxidizable amino acid, have been substituted with a different amino acid. The mutant enzymes of the present invention exhibit altered stability/activity profiles including but not limited to altered **oxidative stability,** altered pH performance profile, altered specific activity and/or altered thermostability.

Brief Summary Text - BSTX (7):

In order to alter the stability or activity profile of amylase enzymes under varying conditions, it has been found that selective replacement, substitution or deletion of oxidizable amino acids, such as a **methionine,** tryptophan, tyrosine, histidine or **cysteine,** results in an altered profile of the variant enzyme as compared to its precursor. Because currently commercially available amylases are not acceptable (stable) under various conditions, there is a need for an amylase having an altered stability and/or activity profile. This altered **stability (oxidative,** thermal or pH performance profile) can be achieved while maintaining adequate enzymatic activity, as compared to the wild-type or precursor enzyme. The characteristic affected by introducing such **mutations** may be a change in **oxidative stability** while maintaining thermal stability or vice versa. Additionally, the substitution of different amino acids for an oxidizable amino acids in the alpha-amylase precursor sequence or the deletion of one or more oxidizable amino acid(s) may result in altered enzymatic activity at a pH other than that which is considered optimal for the precursor alpha-amylase. In other words, the **mutant** enzymes of the present invention may also have altered pH performance profiles, which may be due to the enhanced **oxidative stability** of the enzyme.

Brief Summary Text - BSTX (10):

Preferably the substitution or deletion of one or more amino acids in the amino acid sequence is due to the replacement or deletion of one or more methionine, tryptophan, cysteine, histidine or tyrosine residues in such sequence, most preferably the residue which is changed is a methionine residue. The oxidizable amino acid residues may be replaced by any of the other 20 naturally occurring amino acids. If the desired effect is to alter the **oxidative stability** of the precursor, the amino acid residue may be substituted with a non-oxidizable amino acid (such as alanine, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, or valine) or another oxidizable amino acid (such as cysteine, methionine, tryptophan, tyrosine or histidine, listed in order of most easily oxidizable to less readily oxidizable). Likewise, if the desired effect is to alter thermostability, any of the other 20 naturally occurring amino acids may be substituted (i.e., cysteine may be substituted for methionine).

Brief Summary Text - BSTX (11):

Preferred **mutants** comprise the substitution of a **methionine** residue equivalent to any of the **methionine** residues found in B. lichenifornis alpha-amylase (+8, +15, +197, +256, +304, +366 and +438). Most preferably the **methionine** to be replaced is a **methionine** at a position equivalent to position +197 or +15 in B. licheniformis alpha-amylase. Preferred substitute amino acids to replace the **methionine** at position +197 are alanine (A), isoleucine (I), threonine (T) or **cysteine** (C). The preferred substitute amino acids at position +15 are leucine (L), threonine(T), asparagine (N), aspartate (D), serine (S), valine (V) and isoleucine (I), although other substitute amino acids not specified above may be useful. Two specifically preferred **mutants** of the present invention are M197T and M15L.

Brief Summary Text - BSTX (13):

The alpha-amylase mutants of the present invention, in general, exhibit altered **oxidative stability** in the presence of hydrogen peroxide and other oxidants such as bleach or peracids, or, more specific, milder oxidants such as chloramine-T. Mutant enzymes having enhanced **oxidative stability** will be useful in extending the shelf life and bleach, perborate, percarbonate or peracid compatibility of amylases used in cleaning products. Similarly, reduced **oxidative stability** may be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity. The mutant enzymes of the present invention may also demonstrate a broadened pH performance profile whereby mutants such as M15L show stability for low pH starch liquefaction and mutants such as M197T show stability at high pH cleaning product conditions. The mutants of the present invention may also have altered thermal stability whereby the mutant may have enhanced stability at either high or low temperatures. It is understood that any change (increase or decrease) in the mutant's enzymatic characteristic(s), as compared to its precursor, may be beneficial depending on the desired end use of the mutant alpha-amylase.

Detailed Description Text - DETX (2):
It is believed that amylases used in starch liquefaction may be subject to some form of inactivation due to some activity present in the starch slurry (see commonly owned U.S. application Ser. Nos. 07/785,624 and 07/785,623 and U.S. Pat. No.5,180,669, issued Jan. 19, 1993, incorporated herein by reference). Furthermore, use of an amylase in the presence of oxidants, such as in bleach or peracid containing detergents, may result in partial or complete inactivation of the amylase. Therefore, the preset invention focuses on altering the oxidative sensitivity of amylases. The mutant enzymes of the present invention may also have an altered pH profile and/or altered thermal **stability which may be due to the enhanced oxidative stability** of the enzyme at low or high pH's.

Detailed Description Text - DETX (7):
Specific residues corresponding to positions M197, M15 and W138 of Bacillus licheniformis alpha-amylase are identified herein for substitution or deletion, as are all **methionine,** histidine, tryptophan, **cysteine** and tyrosine positions. The amino acid position number (i.e., +197) refers to the number assigned to the mature Bacillus lichenifonnis alpha-amylase sequence presented in FIG. 2. The invention, however, is not limited to the **mutation** of this particular mature alpha-amylase (B. licheniformis) but extends to precursor alpha-amylases containing amino acid residues at positions which are equivalent to the particular identified due in B. licheniformis alpha-amylase. A residue (amino acid) of a precursor alpha-amylase is equivalent to a residue of B. licheniformis alpha-amylase if it is either homologous (i.e., corresponding in position in either primary or tertiary structure) or analogous to a specific residue or portion of that residue in B. licheniformis alpha-amylase (i.e., having the same or similar functional capacity to combine, react, or interact chemically or structurally).

Detailed Description Text - DETX (14):
Based on the conditions of a preferred liquefaction process, as described in commonly owned U.S. application Ser. Nos. 071788,624 and 071785,623 and U.S. Pat. No. 5,180,669, namely low pH, high temperature and potential oxidation conditions, preferred mutants of the present invention for use in liquefaction processes comprise mutants exhibiting altered pH performance profiles (i.e., low pH profile, pH <6 and preferably pH <5.5), and/or altered thermal stability (i.e., high temperature, about 900.degree.-110.degree. C.), and/or altered **oxidative stability (i.e., enhanced oxidative stability**).

Detailed Description Text - DETX (65):
As can be seen in FIG. 9, there was a wide range of amylase activity (measured in the soluble substrate assay) expressed by the M197X (A4 form) variants. The amylases were partially purified from the supernatants by precipitation with two volumes of ethanol and resuspension. They were then screened for thermal stability (FIG. 10) by heating at 95.degree. C. for 5 minutes in 10 mM acetate buffer pH 5.0, in the presence of 5 mM calcium chloride; the A4 wild-type retained 28% of its activity after incubation. For M197W and M197P we were unable to recover active protein from the supernatants.

Upon sequencing, the M197H variant was found to contain a second mutation, N190K. M1 97L was examined in a separate experiment and was one of the lowest thermally stable variants. There appears to be a broad correlation between expression of amylase activity and thermal stability. The licheniformis amylase is restricted in what residues it can accommodate at position 197 in terms of retaining or enhancing thermal stability: cysteine and threonine are preferred for maximal thermal stability under these conditions whereas alanine and isoleucine are of intermediate stability. However, other substitutions at position +197 result in lowered thermal stability which may be useful for other applications. Additionally, different substitutions at +197 may have other beneficial properties, such as altered pH performance profile or altered **oxidative stability**. For example, the M197C variant was found to inactivate readily by air **oxidation but had enhanced thermal stability**. Conversely, compared to the M197L variant, both M197T and M197A retained not only high thermal stability (FIG. 10), but also high activity (Table III), while maintaining resistance to inactivation by peroxide at pH 5 to pH 10 (FIG. 7).

US-PAT-NO: 5763385

DOCUMENT-IDENTIFIER: US 5763385 A

TITLE: Modified .alpha.-amylases having altered calcium binding properties

DATE-ISSUED: June 9, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bott; Richard R.	Burlingame	CA	N/A	N/A
Shaw; Andrew	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 645971

DATE FILED: May 14, 1996

US-CL-CURRENT: 510/392, 435/201 , 435/202 , 435/836

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which a new calcium binding site is modified by chemically or genetically altering residues associated with that calcium binding site. The novel .alpha.-amylases have altered performance characteristics, such as low pH starch hydrolysis performance, stability and activity profiles.

28 Claims, 10 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

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Brief Summary Text - BSTX (8):

In PCT Publication No. WO94/02597, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more methionines are replaced by any amino acid except **cysteine or methionine**.

Brief Summary Text - BSTX (9):

In PCT Publication No. WO94/18314, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more of the **methionine,** tryptophan, **cysteine,** histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Brief Summary Text - BSTX (15):

Despite the advances made in the prior art, a need exists for an .alpha.-amylase which has altered performance, including activity and stability, to facilitate their use in starch liquefaction, detergents for laundry and dishwashing, baking, textile desizing and other standard uses for amylase. Because commercially available amylases are not acceptable under many conditions due to stability and/or activity problems, there is a need for an amylase having altered, and preferably increased, performance profiles under such conditions. For example, high alkalinity and oxidant (bleach) levels associated with detergents or the extreme conditions present during starch liquefaction can result in both destabilization and lack of activity from .alpha.-amylase. Thus, altered performance characteristics such as thermostability, pH **stability, oxidative stability** or calcium stability which can be achieved while also altering, maintaining, or increasing enzymatic activity as compared to the wild-type or precursor enzyme, would be desirable. Similarly, many .alpha.-amylases are known to require the addition of calcium ion for stability. This is undesirable in some applications due to increased processing costs.

Brief Summary Text - BSTX (17):

It is an object of the present invention to provide an .alpha.-amylase having altered performance profiles, e.g., altered pH **stability, alkaline stability, oxidative stability,** thermal stability or enzymatic activity.

Brief Summary Text - BSTX (27):

The modified .alpha.-amylases according to the present invention will provide several important advantages when compared to prior art .alpha.-amylases. For example, one advantage is found in variants having increased activity at low pH and high temperatures typical of common starch liquefaction methods. Another advantage is found in variants having increased high pH and **oxidative stability** which facilitates their use in detergents. Yet another advantage is provided by variants having improved stability in the absence or low concentration of calcium ion. The objects and attendant advantages of the present invention will be made more clear in the following detailed description and examples.

Detailed Description Text - DETX (7):

"Calcium binding site" means a region within .alpha.-amylase which is suitable for and acts to bind a calcium ion in the presence of free calcium. Calcium is generally believed to be required to maintain the structural integrity of .alpha.-amylase under many conditions and the amino acid residues involved in calcium binding have been shown to be highly conserved among the different enzymes (Machius et al., J. Mol. Biol., vol. 246, pp. 545-559 (1995)). According to the present invention, the characteristics of the calcium binding site are altered compared to a wild-type or precursor .alpha.-amylase so as to alter the performance of the .alpha.-amylase. Alteration of the calcium binding site may include reducing or increasing the affinity of the site to bind calcium ion. By altering the performance is intended to mean the **stability (e.g., oxidative** or thermal) or the activity

(e.g., the rate or efficiency with which the .alpha.-amylase hydrolyzes starch substrate) of the enzyme in its various applications.

Detailed Description Text - DETX (30):

The .alpha.-amylases according to the present invention may exhibit altered performance characteristics providing desirable and unexpected results which are useful in the various applications for which .alpha.-amylases are commonly used. For example, .alpha.-amylases according to the present invention which exhibit altered performance characteristics at low pH, including improved thermostability, improved pH **stability and/or improved oxidative stability,** are useful in low pH liquefaction of starch. Enhanced thermostability will be useful in extending the shelf life of products which incorporate them. Enhanced **oxidative stability** or improved performance is particularly desirable in cleaning products, and for extending the shelf life of .alpha.-amylase in the presence of bleach, perborate, percarbonate or peracids used in such cleaning products. To the contrary, reduced thermal **stability or oxidative stability** may be useful in industrial processes which require the rapid and efficient quenching of amylolytic activity. Additionally, a reduced requirement or stronger affinity for calcium would be advantageous in the presence of sequestering components generally found in detergents, i.e., builders.

Detailed Description Text - DETX (31):

The .alpha.-amylase of the present invention is especially useful in starch processing and particularly in starch liquefaction. Conditions present during commercially desirable liquefaction processes characteristically include low pH, high temperature and potential oxidation conditions requiring .alpha.-amylases exhibiting improved low pH performance, improved thermal **stability and improved oxidative stability**. Accordingly, .alpha.-amylases according to the present invention which are particularly useful in liquefaction exhibit improved performance at a pH of less than about 6, preferably less than about 5.5, and more preferably between about 5.0 and 5.5. Additionally, .alpha.-amylases according to the present invention which exhibit increased thermal stability at temperatures of between about 80.degree.-120.degree. C., and preferably between about 100.degree.-110.degree. C., and increased stability in the presence of oxidants will be particularly useful. Preferably, the .alpha.-amylase according to the present invention which is used in liquefaction further comprises a deletion or substitution at one or more of positions M15, V128, H133, W138, N188, A209 and/or M197.

Detailed Description Text - DETX (32):

In another embodiment of the present invention there are provided detergent compositions in either liquid, gel or granular form, which comprise the .alpha.-amylase according to the present invention. Such detergent compositions will particularly benefit from the addition of an .alpha.-amylase according to the present invention which has increased thermal stability to improve shelf-life or increased **oxidative stability** such that the .alpha.-amylase has improved resistance to bleach or peracid compounds commonly present in detergents. Thus, .alpha.-amylase according to the present

invention may be advantageously formulated into known powdered, liquid or gel detergents having a pH of between about 6.5 and about 12.0. A preferred embodiment of the present invention further comprises a deletion or substitution at one or more of positions M15, V128, H133, W138, N188, A209 and/or M197. Detergent compositions comprising the .alpha.-amylase according to the present invention may further include other enzymes such as endoglycosidases, cellulases, proteases, lipases or other amylase enzymes, for example amylase derived from Bacillus stearothermophilus, as is generally known in the art.

Detailed Description Text - DETX (33):

Embodiments of the present invention which comprise a combination of the .alpha.-amylase according to the present invention with protease enzymes preferably include **oxidatively stable** proteases such as those described in U.S. Pat. No. Re 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk), MAXAPEM (Gist-brocades) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease mutants (**oxidatively stable** proteases), and particularly such mutants having a substitution for the methionine at a position equivalent to M222 in Bacillus amyloliquefaciens, are described in U.S. Pat. No. Re 34,606.

US-PAT-NO: 5756714

DOCUMENT-IDENTIFIER: US 5756714 A

TITLE: Method for liquefying starch

DATE-ISSUED: May 26, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Antrim; Richard L.	Solon	IA	N/A	N/A
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A

APPL-NO: 08/ 411038

DATE FILED: March 27, 1995

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application Ser. No. 08/401,325 filed Mar. 9, 1995, now abandoned and which is incorporated herein by reference in its entirety.

US-CL-CURRENT: 536/102, 435/202 , 435/203 , 435/204 , 435/205 , 435/96 , 435/99

ABSTRACT:

According to the invention a method is provided for liquefying starch comprising the steps of treating the starch prior to or simultaneously with liquefying the starch to inactivate and/or remove the enzyme inhibiting composition present in the starch and form treated starch; adding .alpha.-amylase to the treated starch; and reacting the treated starch for a time and at a temperature effective to liquefy the treated starch. Effective means to treat the starch include the addition of a phytate degrading enzyme and heat treatment, optionally followed by filtration or centrifugation, of granular starch or a starch solution.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (16):

In PCT Publication No. WO 94/02597, a **mutant** .alpha.-amylase having improved **xidative stability** is described wherein one or more methionines are replaced by any amino acid except **cysteine or methionine**.

Brief Summary Text - BSTX (17):

In PCT publication No. 94/18314, a **mutant-amylase** having improved **oxidative stability** is described wherein one or more of the **methionine,** tryptophan, **cysteine,** histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

US-PAT-NO: 5741767

DOCUMENT-IDENTIFIER: US 5741767 A

TITLE: Peracid based dishwashing detergent composition

DATE-ISSUED: April 21, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Nicholson; John Richard	Ramsey	NJ	N/A	N/A
Secemski; Isaac Israel	Teaneck	NJ	N/A	N/A
Rick; Deborah Sue	New Milford	NJ	N/A	N/A
Raible; Duane Anthony	Park Ridge	NJ	N/A	N/A

APPL-NO: 08/ 558994

DATE FILED: November 16, 1995

US-CL-CURRENT: 510/220, 134/25.2 , 510/221 , 510/223 , 510/226 , 510/227 , 510/229 , 510/230 , 510/372 , 510/374 , 510/375 , 510/392 , 510/393 , 510/441

ABSTRACT:

A warewashing composition for a machine dishwasher and a method of using it is described. The composition comprises effective amounts of an organic peroxy acid, and an amylase enzyme which, when incubated at 55.degree. C. in a solution of 2 mM sodium citrate, 1 mM epsilon phthalimidoperoxyhexanoic acid in 36 ppm water at pH 8.0, has a half-life of two minutes or greater based on an activity vs. time plot obtained via monitoring color development at 405 nm of solution samples incubated with p-nitrophenyl-.alpha.-D-maltoheptaoside as substrate and gluco amylase and .alpha.-glucosidase as coupled enzymes; and 1% to 75% by weight of a builder. A 1% aqueous solution of the composition must have a pH of less than 10.

29 Claims, 0 Drawing figures

Exemplary Claim Number: 1,16

---------- KWIC ---------

Brief Summary Text - BSTX (37):

Such .alpha.-amylase enzymes with improved **oxidation stability** and bleach resistance useful in the invention are described in WO 94/02597 (Novo); WO 94/14951 (Novo) and EP 208 491 (Genencor International Inc.) herein incorporated by reference.

Brief Summary Text - BSTX (40):

The .alpha.-amylase is a **mutated** amylase wherein one or more **methionine** amino acid residues is exchanged with an amino acid residue except for **cysteine or methionine**.

US-PAT-NO: 5736499

DOCUMENT-IDENTIFIER: US 5736499 A

TITLE: Mutant A-amylase

DATE-ISSUED: April 7, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Requadt; Carol Ann	Tiburon	CA	N/A	N/A
Ropp; Traci Helen	San Francisco	CA	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A

APPL-NO: 08/ 468700

DATE FILED: June 6, 1995

US-CL-CURRENT: 510/392, 435/201 , 435/202 , 435/203 , 435/204 , 510/393 , 510/530

ABSTRACT:

Novel .alpha.-amylase enzymes are disclosed in which one or more asparagine residues are substituted with a different amino acid or deleted. The disclosed .alpha.-amylase enzymes show altered or improved low pH starch hydrolysis performance, stability and activity profiles.

25 Claims, 17 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

---------- KWIC ---------

Brief Summary Text - BSTX (16):

In PCT Publication No. WO 94/02597, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more methionines are replaced by any amino acid except **cysteine or methionine**.

Brief Summary Text - BSTX (17):

In PCT publication No. WO 94/18314, a **mutant** .alpha.-amylase having improved **xidative stability** is described wherein one or more of the **methionine,** tryptophan, **cysteine,** histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

Brief Summary Text - BSTX (20):

Despite the advances made in the prior art, a need exists for an .alpha.-amylase which is effective enough at low pH values to allow commercial liquefaction at lower pH than currently practical. Similarly, a need exists in the art for a method which allows efficient liquefaction of dry milled grain at high temperatures. Further, a need exists in the art for a method which allows the efficient liquefaction of starch with a decreased reliance on the costly addition of calcium. Additionally, a need exists for a more efficient enzyme to effect a more complete hydrolysis of starch at the liquefaction stage to ensure efficient saccharification. Because commercially available amylases are not acceptable under many conditions due to stability problems, for example, the high alkalinity and oxidant (bleach) levels associated with detergents, there is a need for an amylase having altered, and preferably increased, performance profiles under such conditions. Thus, altered performance characteristics such as increased activity, thermostability, pH **stability, oxidative stability** or calcium stability which can be achieved while also altering, maintaining, or increasing enzymatic activity as compared to the wild type or precursor enzyme, would be desirable.

Brief Summary Text - BSTX (22):

It is an object of the present invention to provide an .alpha.-amylase having altered performance profiles, such as pH **stability, alkaline stability, oxidative stability** or enzymetic activity.

Detailed Description Text - DETX (16):

The .alpha.-amylases according to the present invention exhibit altered performance characteristics providing desirable and unexpected results which are useful in the various applications for which .alpha.-amylases are commonly used. For example, .alpha.-amylases according to the present invention which exhibit altered performance characteristics at low pH, including improved thermostability, improved pH **stability and/or improved oxidative stability,** are useful in low pH liquefaction of starch. Enhanced thermostability will be useful in extending the shelf life of products which incorporate them. Enhanced oxiderive stability or improved performance is particularly desirable in cleaning products, and for extending the shelf life of .alpha.-amylase in the presence of bleach, perborate, percarbonate or peracids used in such cleaning products. To the contrary, reduced thermal **stability or oxidative stability** may be useful in industrial processes which require the rapid and efficient quenching of amylolytic activity.

Detailed Description Text - DETX (17):

The .alpha.-amylase of the present invention is especially useful in starch processing and particularly in starch liquefaction. Conditions present during commercially desirable liquefaction processes characteristically include low pH, high temperature and potential oxidation conditions requiring .alpha.-amylases exhibiting improved low pH performance, improved thermal **stability and improved xidative stability**. Accordingly, .alpha.-amylases according to the present invention which are particularly useful in

liquefaction exhibit improved performance at a pH of less than about 6, preferably less than about 5.5, and most preferably between about 5.0 and 5.5. Additionally, .alpha.-amylases according to the present invention which exhibit increased thermal stability at temperatures of between about 80.degree.-120.degree. C., and preferably between about 100.degree.-110.degree. C., and increased stability in the presence of oxidants will be particularly useful. Preferably, the .alpha.-amylase according to the present invention which is used in liquefaction, in addition to the deletion or substitution of an asparagine, further comprises a deletion or substitution at a methionine or a tryptophan, and particularly at position M15, W138 and/or M197. In a more preferred embodiment, .alpha.-amylase used in starch liquefaction according to the present invention comprises a deletion or substitution at position N188. Most preferably, the amylase is derived from Bacillus lichenformis or Bacillus stearothermophilus and comprises a substitution corresponding to M15T/N188S.

Detailed Description Text - DETX (20):

In another embodiment of the present invention there are provided detergent compositions in either liquid, gel or granular form, which comprise the .alpha.-amylase according to the present invention. Such detergent compositions will particularly benefit from the addition of an .alpha.-amylase according to the present invention which has increased thermal stability to improve shelf-life or increased **oxidative stability** such that the .alpha.-amylase has improved resistance to bleach or peracid compounds commonly present in detergents. Thus, .alpha.-amylase according to the present invention may be advantageously formulated into known powdered, liquid or gel detergents having a pH of between about 6.5 and about 12.0. A preferred embodiment of the present invention further comprises the deletion or substitution of a methionine residue or a tryptophan residue, for example M15, M197 or W138 as described in commonly assigned U.S. patent application Ser. Nos. 08/289,351 and 08/409,771, the disclosures of which are incorporated by reference. Also preferably, an .alpha.-amylase according to the present invention used in detergent compositions comprises a deletion or substitution at position N188. Detergent compositions comprising the .alpha.-amylase according to the present invention may further include other enzymes such as endoglycosidases, cellulases, proteases, lipases or other amylase enzymes, particularly .alpha.-amylase derived from Bacillus stearothermophilus as well as additional ingredients as generally known in the art.

Detailed Description Text - DETX (21):

Embodiments of the present invention which comprise a combination of the .alpha.-amylase according to the present invention with protease enzymes preferably include **oxidatively stable** proteases such as those described in U.S. Pat. No. Re. 34,606, incorporated herein by reference, as well as commercially available enzymes such as DURAZYM (Novo Nordisk), MAXAPEM (Gist-brocades) and PURAFECT.RTM. OxP (Genencor International, Inc.). Methods for making such protease mutants (**oxidatively stable** proteases), and particularly such mutants having a substitution for the methionine at a position equivalent to M222 in Bacillus amyloliquefaciens, are described in U.S. Pat. No. Re. 34,606.

Detailed Description Text - DETX (24):

The improved .alpha.-amylases according to the present invention provide several important advantages when compared to wild type Bacillus .alpha.-amylases. For example, one advantage is the increased activity found at low pH and high temperatures typical of common starch liquefaction methods. Another advantage is the increased high pH and **oxidative stability** which facilitates their use in detergents. Another advantage is that a more complete hydrolysis of starch molecules is achieved which reduces residual starch in the processing stream. Yet another advantage is their improved stability in the absence of calcium ion. Yet another advantage is that the addition of equal protein doses of .alpha.-amylase according to the invention provide superior performance when compared to wild type Bacillus lichenformis .alpha.-amylase due to improvements in both specific activity and stability under stressed conditions. In other words, because of the generally increased stability of the amylases according to the present invention, the increased specific activity on starch of the inventive amylases translates to even greater potential performance benefits of this variant. Under conditions where the wild type enzyme is being inactivated, not only does more of the inventive amylase survive because of its increased stability, but also that which does survive expresses proportionally more activity because of its increased specific activity.

US-PAT-NO: 5716810

DOCUMENT-IDENTIFIER: US 5716810 A

TITLE: Nucleic acid encoding the mature .beta..sub.B chain of inhibin and method for synthesizing polypeptides using such nucleic acid

DATE-ISSUED: February 10, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mason; Anthony J.	San Francisco	CA	N/A	N/A
Seeburg; Peter H.	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 459214

DATE FILED: June 2, 1995

PARENT-CASE:

This is a divisional application of U.S. Ser. No. 08/197,792 filed Feb. 17, 1994, now U.S. Pat. No. 5,525,488, which is a divisional application of U.S. Ser. No. 07/958,414 filed Oct. 8, 1992, now U.S. Pat. No. 5,310,661, which is a divisional application of U.S. Ser. No. 07/744,207 filed Aug. 12, 1991, now U.S. Pat. No. 5,215,893 which is a divisional application of U.S. Ser. No. 07/215,466 filed Jul. 5, 1988, now U.S. Pat. No. 5,089,396, which is a divisional of U.S. Ser. No. 06/906,729, filed Dec. 31, 1986, now U.S. Pat. No. 4,798,885, which is a continuation-in-part application of U.S. Ser. No. 06/827,710 filed Feb. 7, 1986, now abandoned, which is a continuation-in-part application of U.S. Ser. No. 06/783,910 filed Oct. 3, 1985, now abandoned.

US-CL-CURRENT: 435/69.4, 435/252.3 , 435/325 , 435/69.1 , 536/23.1 , 536/23.5 , 536/23.51

ABSTRACT:

DNA encoding the prepro inhibin .alpha. and .beta. chains has been isolated. This DNA is ligated into expression vectors and used to transform host cells for the preparation of inhibin or activin. Also provided are prohormone domains and other inhibin .alpha. or .beta. chain derivatives having therapeutic or diagnostic interest. The compositions provided herein are useful in the manipulation of fertility in animals.

20 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

---------- KWIC ---------

Detailed Description Text - DETX (16):

Insertional and deletional amino acid sequence variants are proteins in which one or more amino acid residues are introduced into or removed from a predetermined site in the target inhibin, activin, prodomain or proform of inhibin or activin. Most commonly, insertional variants are fusions of heterologous proteins or polypeptides to the amino or carboxyl terminus of the .alpha. or .beta. chains, the prodomains or other inhibin derivatives. Immunogenic derivatives are made by fusing an immunogenic polypeptide to the target sequence, e.g. a prodomain polypeptide, by synthesis in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Such immunogenic polypeptides preferably are bacterial polypeptides such as trpLE, beta-galactosidase and the like, together with their immunogenic fragments. Other insertions entail inserting heterologous eukaryotic (e.g. the herpes virus gD signal) or microbial secretion signal or protease processing sequences upstream from the NH.sub.2 -terminus of the protein to be secreted. Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **oxidative stability** of the .alpha. or .beta. chain. Deletional derivatives will produce .alpha. or .beta. chain fragments. Such fragments, when biologically or immunologically active, are within the scope herein. For instance, a fragment comprising .beta..sub.B or .beta..sub.A residues about from 11 to 45 (numbered from mature Gly.sub.1) is to be included within the scope herein.

Detailed Description Text - DETX (18):

Substitution derivatives are produced by **mutating** the DNA in a target codon, so that thereafter a different amino acid is encoded by the codon, with no concomitant change in the number of residues present in the molecule expressed from **mutated** DNA. Substitutions or deletions are useful for example in increasing the stability of the proteins herein by eliminating proteolysis sites, wherein resides are substituted within or adjacent to the sites or are deleted from the sites, or by introducing additional disulfide bond through the substitution of **cysteine** for other residues. Substitution are useful for facilitating the synthesis or recovery of mature or prodomain .alpha. or .beta. chains. For example, **methionine** residues within the mature inhibin sequences are substituted or deleted, propro sequences deleted, **methionine** is inserted at the -1 site immediately NH.sub.2 terminal to the mature NH.sub.2 terminal residue and another sequence inserted N-terminal to the exogenous **methionine**. The inhibin derivative in this case is expressed as a fusion having an intermediate methionyl residue, which in turn is cleaved at this residue by cyanogen bromide in accordance with known practice. The mature inhibin derivative released from the fusion is recovered.

US-PAT-NO: 5665568

DOCUMENT-IDENTIFIER: US 5665568 A

TITLE: Nucleic acid encoding the mature .beta..sub.A chain of inhibin and method for synthesizing polypeptides using such nucleic acid

DATE-ISSUED: September 9, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mason; Anthony J.	San Francisco	CA	N/A	N/A
Seeburg; Peter H.	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 459850

DATE FILED: June 2, 1995

PARENT-CASE:

This is a divisional application of U.S. Ser. No. 08/197,792, filed Feb. 17, 1994, now U.S. Pat. No. 5,525,488, which is a divisional application of U.S. Ser. No. 07/958,414, filed Oct. 8, 1992, now U.S. Pat. No. 5,310,661, which is a divisional application of U.S. Ser. No. 07/744,207, filed Aug. 12, 1991, now U.S. Pat. No. 5,215,893, which is a divisional application of U.S. Ser. No. 07/215,466, filed Jul. 5, 1988, now U.S. Pat. No. 5,089,396, which is a divisional of U.S. Ser. No. 06/906,729, filed Dec. 31, 1986, now U.S. Pat. No. 4,798,885, which is a continuation-in-part application of U.S. Ser. No. 06/827,710, filed Feb. 7, 1986, now abandoned, which is a continuation-in-part application of U.S. Ser. No. 06/783,910, filed Oct. 3, 1985, now abandoned.

US-CL-CURRENT: 435/69.4, 435/252.3 , 435/320.1 , 435/325 , 435/350 , 435/352 , 435/354 , 435/360 , 435/364 , 435/365.1 , 435/367 , 435/370 , 435/69.1 , 536/23.1 , 536/23.5 , 536/23.51

ABSTRACT:

DNA encoding the prepro inhibin .alpha. and .beta. chains has been isolated. This DNA is ligated into expression vectors and used to transform host cells for the preparation of inhibin or activin. Also provided are prohormone domains and other inhibin .alpha. or .beta. chain derivatives having therapeutic or diagnostic interest. The compositions provided herein are useful in the manipulation of fertility in animals.

30 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

---------- KWIC ---------

Detailed Description Text - DETX (16):

Insertional and deletional amino acid sequence variants are proteins in which one or more amino acid residues are introduced into or removed from a predetermined site in the target inhibin, activin, prodomain or proform of inhibin or activin. Most commonly, insertional variants are fusions of heterologous proteins or polypeptides to the amino or carobyxl terminus of the .alpha. or .beta. chains, the prodomains or other inhibin derivatives. Immunogenic derivatives are made by fusing an immunogenic polypeptide to the target sequence, e.g. a prodomain polypeptide, by synthesis in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Such immunogenic polypepetides preferably are bacterial polypeptides such as trpLE, beta-galactosidase and the like, together with their immunogenic fragments. Other insertions entail inserting heterologous eukaryotic (e.g. the herpes virus gD signal) or microbial secretion signal or protease processing sequences upstream from the NH.sub.2 -terminus of the protein to be secreted. Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **oxidative stability** of the .alpha. or .beta. chain. Deletional derivatives will produce .alpha. or .beta. chain fragments. Such fragments, when biologically or immunologically active, are within the scope herein. For instance, a fragment comprising .beta..sub.B or .beta..sub.A residues about from 11 to 45 (numbered from mature Gly.sub.1) is to be included within the scope herein.

Detailed Description Text - DETX (18):

Substitution derivatives are produced by **mutating** the DNA in a target codon, so that thereafter a different amino acid is encoded by the codon, with no concomitant change in the number of residues present in the molecule expressed from the **mutated** DNA. Substitutions or deletions are useful for example in increasing the stability of the proteins herein by eliminating proteolysis sites, wherein residues are substituted within or adjacent to the sites or are deleted from the sites, or by introducing additional disulfide bonds through the substitution of **cysteine** for other residues. Substitutions are useful for facilitating the synthesis or recovery of mature or prodomain .alpha. or .beta. chains. For example, **methionine** residues within the mature inhibin sequences are substituted or deleted, prepro sequences deleted, **methionine** is inserted at the -1 site immediately NH.sub.2 terminal to the mature NH.sub.2 terminal residue and another sequence inserted N-terminal to the exogenous **methionine**. The inhibin derivative in this case is expressed as a fusion having an intermediate methionyl residue, which in turn is cleaved at this residue by cyanogen bromide in accordance with known practice. The mature inhibin derivative released from the fusion is recovered.

US-PAT-NO: 5652127

DOCUMENT-IDENTIFIER: US 5652127 A

TITLE: Method for liquefying starch

DATE-ISSUED: July 29, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mitchinson; Colin	Half Moon Bay	CA	N/A	N/A
Solheim; Leif P.	Clinton	IA	N/A	N/A

APPL-NO: 08/ 459984

DATE FILED: June 2, 1995

US-CL-CURRENT: 435/99, 435/201 , 435/202 , 435/203 , 435/204 , 435/205 , 435/275 , 435/72

ABSTRACT:

According to the invention a method is provided for liquefying starch comprising the steps of adding a sodium composition to the starch prior to or simultaneously with liquefying the starch; adding .alpha.-amylase to the treated starch; and reacting the treated starch for a time and at a temperature effective to liquefy the treated starch. Preferred sodium compositions comprise sodium chloride, sodium bicarbonate, sodium benzoate, sodium sulfate, sodium bisulfite, sodium ascorbate, sodium acetate, sodium nitrate, sodium tartrate, sodium tetraborate, sodium propionate, sodium citrate, sodium succinate, monosodium glutamate, trisodium citrate, sodium phosphate or a mixture thereof.

10 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Brief Summary Text - BSTX (15):

In PCT Publication No. WO 94/02597, a **mutant** .alpha.-amylase having improved **oxidative stability** is described wherein one or more methionines are replaced by any amino acid except **cysteine or methionine**.

Brief Summary Text - BSTX (16):

In PCT publication No. 94/18314; a **mutant-amylase** having improved **oxidative stability** is described wherein one or more of the **methionine,** tryptophan,

cysteine, histidine or tyrosine residues is replaced with a non-oxidizable amino acid.

US-PAT-NO: 5543498

DOCUMENT-IDENTIFIER: US 5543498 A

TITLE: Peptides to overcome inhibition of nerve growth

DATE-ISSUED: August 6, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fishman; Mark C.	Newton Centre	MA	N/A	N/A
Igarashi; Michihiro	Saitama	N/A	N/A	JP
Strittmatter; Stephen M.	Clinton	CT	N/A	N/A

APPL-NO: 08/ 417279

DATE FILED: April 5, 1995

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation of application Ser. No. 08/166,350, filed Dec. 14, 1993, abandoned, which is a continuation-in-part of application Ser. No. 08/162,480, filed Dec. 7, 1993, abandoned. The content of all the aforesaid applications are relied upon and incorporated by reference in their entirety.

US-CL-CURRENT: 530/328

ABSTRACT:

Inhibition of nerve growth normally helps to prevent aberrant pathway or target selection, but also prevents needed regeneration in the mammalian central nervous system. The responsible inhibitory ligands are unknown, but pertussis toxin-sensitive G proteins, which are enriched in growth cones, appear to be involved in causing the responding growth cones to collapse. GAP-43 is an intracellular protein that can amplify the response to the stimulation of G protein-coupled receptors. We have attempted to modify the sensitivity of nerves to inhibitory signals by the use of GAP-43 peptides. The peptide corresponding to the native amino terminus sequence stimulates G.sub.o and enhances the growth cone collapse induced by inhibitory ligands. Modification of two critical cysteines generates peptides which inhibit G.sub.o and which markedly reduce the degree of inhibitor-mediated growth cone collapse.

4 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Drawing Description Text - DRTX (6):

FIG. 3(A): The effect of **mutant** decapeptides upon GTP.sub..gamma. S binding to G.sub.o. In each case the single letter indicates the amino acid substituted for Cys.sup.3 and Cys.sup.4. The concentration of each peptide was 250 .mu.M. The activity of GTP.sub..gamma. S-binding to G.sub.o protein without peptides is shown as 100%. The sequence of native N-terminus decapeptide of rat GAP-43 (1-10 peptide) is MLCCMRRTKQ [SEQ ID NO: 1]. Both **cysteine** residues at positions 3 and 4 in the native 1-10 peptide were replaced with **methionine** (M) [SEQ ID NO: 2], tyrosine (Y) [SEQ ID NO: 3], aspartate (D) [SEQ ID NO: 4], glutamate (E) [SEQ ID NO: 5], lysine (K) [SEQ ID NO: 6], arginine (R) [SEQ ID NO: 7], serine (S) [SEQ ID NO: 8], and tryptophan (W) [SEQ ID NO: 9]. The values shown are the means .+-.S.E.M. for three separate experiments.

Detailed Description Text - DETX (6):

The above data posed the question of whether similar peptides could be designed that would interfere with G protein signaling, thereby inhibiting growth cone collapse. It had previously been found that the amino terminal decapeptide of GAP-43, if stored without dithiothreitol (DTT), acquires the ability to inhibit G.sub.o (S. M. Strittmatter et al., J. Biol. Chem. 266:22465 (1991)), suggesting that oxidation or other modification of the cysteines could change the activity of the peptide. As shown in FIGS. 2A and B, oxidation of the peptide by performic acid renders it inhibitory for G.sub.o. The sensitivity to oxidation presumably explains why the GAP-43 1-10 peptide stored without DTT is inhibitory to G.sub.o, whereas that stored with DTT is stimulatory (Y. Sudo et al., EMBO J. 11:2095 (1992)). It was felt that **oxidation might be insufficiently stable** for the examination of this peptide's bioactivity, since the interior of the cell is a reducing environment (C. Hwang et al., Science 257:1496 (1992)). Therefore, several peptides with different amino acids substituted for the two cysteines were synthesized. The preparation of these peptides, or their functional derivatives, can be achieved by employing well known techniques in the field of peptide chemistry. For example, the Merrifield procedure for solid-state peptide synthesis can be used (B. Gutte and R. B. Merrifield, J. Biol. Chem. 246(6):1922 (1971)). This procedure involves attaching a t-Butoxycarbonyl protected amino acid to a solid polystyrene resin, removal of the amino protecting group, and forming a peptide linkage between the resin bound amino acid and a second protected amino acid via a carbodiimide mediated condensation. This procedure is repeated with the appropriate amino acids until the desired peptide has been synthesized. Other techniques and reagents for the preparation of peptides are well known in the art, and are set forth, for example, in Bodanszky, M., et al., The Practice of Peptide Synthesis, Springer-Verlag, publisher, New York, N.Y. (1984), and in Bodanszky, M., The Principles of Peptide Synthesis, Springer-Verlag, publisher, New York, N.Y. (1984).

Detailed Description Text - DETX (90):

Both **cyst ine** residues at positions 3 and 4 in the native GAP-43 1-10 peptide were replaced with **methionine,** tyrosine, aspartate, glutamate, lysine, arginine, serine, and tryptophan. These **mutant** peptides were tested for their ability to influence GTP.sub..gamma. S binding to G.sub.o.

US-PAT-NO: 5525488

DOCUMENT-IDENTIFIER: US 5525488 A
See image for Certificate of Correction

TITLE: Nucleic acid encoding the mature .alpha. chain of inhibin and method for synthesizing polypeptides using such nucleic acid

DATE-ISSUED: June 11, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mason; Anthony J.	San Francisco	CA	N/A	N/A
Seeburg; Peter H.	San Francisco	CA	N/A	N/A

APPL-NO: 08/ 197792

DATE FILED: February 17, 1994

PARENT-CASE:

This is a divisional application of U.S. Ser. No. 07/958,414 filed Oct. 8, 1992, now U.S. Pat. No. 5,310,661, which is a divisional application of U.S. Ser. No. 07/744,207 filed Aug. 12, 1991, now U.S. Pat. No. 5,215,893, which is a divisional application of U.S. Ser. No. 07/215,466 filed Jul. 5, 1988, now U.S. Pat. No. 5,089,396, which is a divisional application of U.S. Ser. No. 06/906,729 filed Dec. 31, 1986, now U.S. Pat. No. 4,798,885, which is a continuation-in-part application of U.S. Ser. No. 06/827,710 filed Feb. 7, 1986, now abandoned, which is a continuation-in-part application of U.S. Ser. No. 06/783,910 filed Oct. 3, 1985, now abandoned.

US-CL-CURRENT: 435/69.4, 435/252.3 , 435/254.11 , 435/320.1 , 435/360 , 536/23.5

ABSTRACT:

DNA encoding the prepro inhibin .alpha. and .beta. chains has been isolated. This DNA is ligated into expression vectors and used to transform host cells for the preparation of inhibin or activin. Also provided are prohormone domains and other inhibin .alpha. or .beta. chain derivatives having therapeutic or diagnostic interest. The compositions provided herein are useful in the manipulation of fertility in animals.

17 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

---------- KWIC ---------

Detailed Description Text - DETX (16):

Insertional and deletional amino acid sequence variants are proteins in which one or more amino acid residues are introduced into or removed from a predetermined site in the target inhibin, activin, prodomain or proform of inhibin or activin. Most commonly, insertional variants are fusions of heterologous proteins or polypeptides to the amino or carboxyl terminus of the .alpha. or .beta. chains, the prodomains or other inhibin derivatives. Immunogenic derivatives are made by fueling an immunogenic polypeptide to the target sequence, e.g. a prodomain polypeptide, by synthesis in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Such immunogenic polypeptides preferably are bacterial polypeptides such as trpLE, beta-galactosidase and the like, together with their immunogenic fragments. Other insertions entail inserting heterologous eukaryotic (e.g. the herpes virus gD signal) or microbial secretion signal or protease processing sequences upstream from the NH.sub.2 -terminus of the protein to be secreted. Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **oxidative stability** of the .alpha. or .beta. chain. Deletional derivatives will produce .alpha. or .beta. chain fragments. Such fragments, when biologically or immunologically active, are within the scope herein: For instance, a fragment comprising .beta..sub.B or .beta..sub.A residues about from 11 to 45 (numbered from mature Gly.sub.1) is to be included within the scope herein.

Detailed Description Text - DETX (18):

Substitution derivatives are produced by **mutating** the DNA in a target codon, so that thereafter a different amino acid is encoded by the codon, with no concomitant change in the number of residues present in the molecule expressed from the **mutated** DNA. Substitutions or deletions are useful for example in increasing the stability of the proteins herein by eliminating proteolysis sites, wherein residues are substituted within or adjacent to the sites or are deleted from the sites, or by introducing additional disulfide bonds through the substitution of **cysteine** for other residues. Substitutions are useful for facilitating the synthesis or recovery of mature or prodomain .alpha. or .beta. chains. For example, **methionine** residues within the mature inhibin sequences are substituted or deleted, prepro sequences deleted, **methionine** is inserted at the -1 site immediately NH.sub.2 terminal to the mature NH.sub.2 terminal residue and another sequence inserted N-terminal to the exogenous **methionine**. The inhibin derivative in this case is expressed as a fusion having an intermediate methionyl residue, which in turn is cleaved at this residue by cyanogen bromide in accordance with known practice. The mature inhibin derivative released from the fusion is recovered.

US-PAT-NO: 5514582

DOCUMENT-IDENTIFIER: US 5514582 A

TITLE: Recombinant DNA encoding hybrid immunoglobulins

DATE-ISSUED: May 7, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Capon; Daniel J.	San Mateo	CA	N/A	N/A
Lasky; Laurence A.	Sausalito	CA	N/A	N/A

APPL-NO: 08/ 185670

DATE FILED: January 21, 1994

PARENT-CASE:

This is a continuation of application Ser. No. 07/986,931 filed on Dec. 8, 1992, now U.S. Pat. No. 5,428,130 which is a continuation of application Ser. No. 07/808,122 filed Dec. 16, 1991, now U.S. Pat. No. 5,225,538, which is a division of application Ser. No. 07/440,625 filed Nov. 22, 1989, now U.S Pat. No. 5,116,964, which is a continuation-in-part of application Ser. No. 07/315,015 filed Feb. 23, 1989, now U.S. Pat. No. 5,098,833.

US-CL-CURRENT: 435/252.3, 435/320.1 , 435/69.7 , 536/23.5 , 536/23.52 , 536/23.53

ABSTRACT:

Novel polypeptides are provided, together with methods for making and using them, and nucleic acids encoding them. These polypeptides are useful as cell surface adhesion molecules and ligands, and are useful in therapeutic or diagnostic compositions and methods.

19 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

---------- KWIC ---------

Detailed Description Text - DETX (78):

Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **oxidative stability** of the LHR. Deletion or substitutions of potential proteolysis sites, e.g. Arg Arg, is accomplished by

deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

Detailed Description Text - DETX (223):

Correct **mutants** were tested for expression by transfection onto human kidney 293 cells using previously described methods. 35S **methionine and cysteine** labeled supernatants were analyzed by immunoprecipitation with protein A sepharose beads in the absence of added antibodies. The precipitated proteins were analyzed on 7.5% polyacrylamide-SDS gels either with or without reduction with beta mercaptoethanol. Plasmids that resulted in correctly expressed chimeras were introduced into 293 cells by transfection in the presence of selective plasmids encoding resistance to G418 as well as dihydrofolate reductase. Clones were selected in G418, and the incorporated plasmids were amplified in the presence of methotrexate. Permanent cell lines expressing high levels of each construct were grown to large scale in T-flasks, and the cell supernatants were clarified by centrifugation and filtration. The resultant supernatants were concentrated by Amicon filtration and passed over standard protein A-sepharose columns, washed with PBS, and eluted with 0.1M Acetic Acid, 0.15M NaCl (pH 3.5). The eluted material was immediately neutralized with 3M Tris, pH 9, and quantitated by SDS gel electrophoresis as well as an ELISA assay.

US-PAT-NO: 5500364

DOCUMENT-IDENTIFIER: US 5500364 A
See image for Certificate of Correction

TITLE: Bacillus lentus alkaline protease varints with enhanced stability

DATE-ISSUED: March 19, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Christianson; Teresa	Cotati	CA	N/A	N/A
Goddette; Dean	Rohnert Park	CA	N/A	N/A
Ladin; Beth F.	Santa Rosa	CA	N/A	N/A
Lau; Maria R.	Fairfield	CA	N/A	N/A
Paech; Christian	Santa Rosa	CA	N/A	N/A
Reynolds; Robert B.	Santa Rosa	CA	N/A	N/A
Wilson; Charles R.	Santa Rosa	CA	N/A	N/A
Yang; Shiow-Shong	Santa Rosa	CA	N/A	N/A

APPL-NO: 08/ 254021

DATE FILED: June 2, 1994

PARENT-CASE:

This is a divisional application of U.S. application Ser. No. 07/706,691 filed on May 29, 1995, now U.S. Pat. No. 5,340,735.

US-CL-CURRENT: 435/221, 435/220 , 435/222 , 435/252.3 , 435/252.31 , 435/69.1 , 536/23.2

ABSTRACT:

Mutant B. lentus DSM 5483 proteases are derived by the replacement of at least one amino acid residue of the mature form of the B. lentus DSM 5483 alkaline protease. The mutant proteases are expressed by genes which are mutated by site-specific mutagenesis. The amino acid sites selected for replacement are identified by means of a computer based method which compares the three dimensional structure of the wild-type protease and a reference protease.

54 Claims, 18 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

---------- KWIC ---------

Brief Summary Text - BSTX (5):

Subtilisins are a family of extracellular proteins having molecular weights in the range of 25,000-35,000 daltons and are produced by various Bacillus species. These proteins function as peptide hydrolases in that they catalyze the hydrolysis of peptide linkages in protein substrates at neutral and alkaline pH values. Subtilisins are termed serine proteases because they contain a specific serine residue which participates in the catalytic hydrolysis of peptide substrates. A subtilisin enzyme isolated from soil samples and produced by Bacillus lentus for use in detergent formulations having increased protease and **oxidative stability** over commercially available enzymes under conditions of pH 7 to 10 and at temperature of 10.degree. to 60.degree. C. in aqueous solutions has been disclosed in copending patent application Ser. No. 07/398,854, filed on Aug. 25, 1989. This B. lentus alkaline protease enzyme (BLAP, vide infra) is obtained in commercial quantities by cultivating a Bacillus licheniformis ATCC 53926 strain which had been transformed by an expression plasmid which contained the wild type BLAP gene and the B. licheniformis ATCC 53926 alkaline protease gene promoter.

Brief Summary Text - BSTX (6):

Industrial processes generally are performed under physical conditions which require highly stable enzymes. Enzymes may be inactivated by high temperatures, pH extremes, oxidation, and surfactants. Even though Bacillus subtilisin proteases are currently used in many industrial applications, including detergent formulations, stability improvements are still needed. Market trends are toward more concentrated detergent powders, and an increase in liquid formulations. Increased shelf **stability and oxidative stability,** with retention of catalytic efficiency are needed. It is therefore desirable to isolate novel enzymes with increased stability, or to improve the stability of existing enzymes, including subtilisin proteases such as BLAP.

Brief Summary Text - BSTX (16):

EP 0251446 teaches the construction of mutant carbonyl hydrolases (proteases) which have at least one property different from the parental carbonyl hydrolase. It describes mutations which effect (either improve or decrease) **oxidative stability,** substrate specificity, catalytic activity, thermal stability, alkaline stability, pH activity profile, and resistance to autoproteolysis. These mutations were selected for introduction into Bacillus amyloliquefaciens subtilisin BPN' after alignment of the primary sequences of BPN+ and proteases from B. subtilis, B. licheniformis, and thermitase. Such alignment can then be used to select amino acids in these other proteases which differ, as substitutes for the equivalent amino acid in the B. amyloliquefaciens carbonyl hydrolase. This application also describes alignment on the basis of a 1.8 .ANG. X-ray crystal structure of the B. amyloliquefaciens protease. Amino acids in the carbonyl hydrolase of B. amyloliquefaciens which when altered can affect stability, substrate specificity, or catalytic efficiency include: Met50, Met124, and Met222 for **oxidative stability**; Tyr104, Ala152, Glu156, Gly166, Gly169, Phe189, and Tyr217 for substrate specificity; N155 alterations were found to decrease turnover,

and lower Km; Asp36, Ile 107, Lys170, Asp197, Ser204, Lys213, and Met222 for alkaline stability; and Met199, and Tyr21 for thermal stability. Alteration of other amino acids was found to affect multiple properties of the protease. Included in this category are Ser24, Met50, Asp156, Gly166, Gly169, and Tyr217. Substitution at residues Ser24, Met50, Ile107, Glu156, Gly166, Gly169, Ser204, Lys213, Gly215, and Tyr217 was predicted to increase thermal and alkaline stability. An important point about this patent application is that with the exception of those mutations effecting substrate specificity, no rational mutational approach for improving the alkaline or temperature stability of a protease based upon computer simulations of an X-ray crystal structure is described.

Brief Summary Text - BSTX (20):

Sensitivity to oxidation is an important deficiency of serine proteases used in detergent applications (Stauffer, C. E., and Etson, D. (1969) J. Biol. Chem. 244:5333-5338). EP 0130756, EP 0247647, and U.S. Pat. No. 4,760,025 teach a saturation **mutation** method where one or multiple **mutations** are introduced into the subtilisin BPN' at amino acid residues Asp32, Asn155, Tyr104, Met222, Gly166, His64, Ser221, Gly169, Glu156, Ser33, Phe189, Tyr217, and/or Ala152. Using this approach **mutant** proteases exhibiting improved **oxidative stability,** altered substrate specificity, and/or altered pH activity profiles are obtained. A method is taught in which improved **oxidative stability** is achieved by substitution of **methionine, cysteine,** tryptophan, and lysine residues. These publications also teach that **mutations** within the active site region of the protease are also most likely to influence activity. Random or selected **mutations** can be introduced into a target gene using the experimental approach but neither EP 0130756, EP 0247647, nor U.S. Pat. No. 4,760,025 teach a method for predicting amino acid alterations which will improve the thermal or surfactant stability of the protease.

US-PAT-NO: 5492813

DOCUMENT-IDENTIFIER: US 5492813 A

TITLE: Muteins of .beta.-galactosidase fragments having increased activity

DATE-ISSUED: February 20, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Eisenbeis; Scott J.	Indianapolis	IN	N/A	N/A
Boguslawski; Sophie J.	Indianapolis	IN	N/A	N/A
Krevolin; Mark	Pinole	CA	N/A	N/A
Ledden; David J.	Indianapolis	IN	N/A	N/A

APPL-NO: 08/ 146673

DATE FILED: November 1, 1993

US-CL-CURRENT: 435/7.6, 435/18 , 435/188 , 435/207 , 435/69.1 , 435/963 , 930/240

ABSTRACT:

Muteins of enzyme acceptor polypeptide fragments of .beta.-galactosidase are provided which exhibit substantially increased kinetic complementation activity with no significant loss in stability. A preferred enzyme acceptor fragment has an amino acid other than cysteine located at position 500 of the natural sequence. An especially preferred substitution is serine or valine. Other preferred muteins have an amino acid other than methionine located at position 443, with leucine being especially preferred, or an amino acid other than cysteine at position 76, with leucine being an especially preferred substitution. Also provided are methods for producing the novel muteins, reagent compositions comprising the novel muteins, and immunoassay methods for determining an analyte in which the novel mutein recombines with an enzyme donor polypeptide fragment to form enzymatically active .beta.-galactosidase.

36 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

---------- KWIC ---------

Brief Summary Text - BSTX (13):

Predetermined, site-directed **mutagenesis** of tRNA synthetase in which a

cysteine residue is converted to serine has been reported (G. Winter et al., 1982, Nature, 299, 756-758, and A. Wilkinson et al., 1984, Nature, 307, 187-188). Estell et al., U.S. Pat. No. 4,760,025, issued Jul. 26, 1988 describe a cloned subtilisin gene modified at specific sites to cause amino acid substitutions of certain **methionine** residues. Koths et al., U.S. Pat. No. 4,752,585 issued Jun. 21, 1988 and U.S. Pat. No. 5,116,943, issued May 26, 1992, describe the protection of a therapeutic protein such as interleukin-2 or interferon-.beta. against oxidation by substituting a conservative amino acid for each methionyl residue susceptible to chloramine T or peroxide oxidation.

Brief Summary Text - BSTX (14):

Buchwalter et al., European Appl. No. 91106224.8, published Nov. 27, 1991, describe an animal somatotropin derivative in which **cysteine** has been substituted by site-specific **mutagenesis** techniques for certain serine and tyrosine residues and in which glutamic acid has been substituted for certain **cysteine** residues. Breddam et al., PCT/DK91/00103 published Oct. 31, 1991, describe chemically modified detergent enzymes wherein one or more methionines have been **mutated** into cysteines, and then said cysteines are subsequently chemically modified in order to improve **stability of the enzyme toward oxidative** agents. Mattes et el., U.S. Pat. No. 4,963,469, issued Oct. 16, 1990, describe a change of an amino acid in the region between amino acid 430 and 550 of .beta.-galactosidase to another amino acid to produce an enzymatically inactive, immunologically active .beta.-galactosidase mutein. Estell et al. (1985, J. Biol. Chem. 260, 6518-6521) used site-directed **mutagenesis** to alter the **methionine** 222 residue of subtilisin which is a primary site for oxidative inactivation of the enzyme. These authors found that **mutants** containing non-oxidizable amino acids, i.e., serine, alanine and leucine, were resistant to peroxide inactivation, whereas **methionine and cysteine**-substituted enzymes were rapidly inactivated.

Brief Summary Text - BSTX (24):

The novel muteins of the present invention are conveniently prepared by causing site-directed **mutagenesis** at the appropriate location on the gene coding for the parent enzyme acceptor. Site-directed **mutagenesis** methods (Wallace et al., 1981, Nucleic Acids Res. 9, 3647-3656; Zoller and Smith, 1982, Nucleic Acids Res. 10, 6487-6500; and Deng and Nickoloff, 1992, Anal. Biochem. 200, 81-88) permit the replacement of **cysteine**-500, **methionine**-443 or **cysteine**-76 of .beta.-galactosidase with any amino acid. Chemical synthesis of the polypeptide fragment is not beyond the scope of the present invention; however, such techniques are generally applied to the preparation of polypeptides that are relatively short in amino acid length.

Detailed Description Text - DETX (2):

In accordance with the present invention, the novel enzyme acceptor polypeptide fragments of .beta.-galactosidase are prepared by site-directed **mutagenesis** methods, wherein a particular location on the gene coding for an enzyme acceptor fragment is **mutagenized**. In one embodiment of the present invention, site-directed **mutagenesis** methods are used to cause a **mutation** at

the location coding for **cysteine** at position 500 in the natural sequence, thereby causing the substitution of a conservative amino acid for **cysteine**. A preferred amino acid substitution is valine or serine. Other amino acids may also be substituted, but conservative substitutions are preferred. By conservative substitution is meant replacement of an amino acid of .beta.-galactosidase by an amino acid which has similar characteristics and which is not likely to have an adverse effect on either the enzyme acceptor's ability to complement with enzyme donor or on the catalytic activity of the reformed .beta.-galactosidase. Examples of such conservative amino acid substitutions are glycine, alanine, valine, leucine, isoleucine, serine, threonine and **methionine**. An especially preferred substitution for **cysteine** is serine or valine, and an especially preferred parent enzyme acceptor is EA22, which is described fully in U.S. Pat. No. 4,708,929.

Claims Text - CLTX (11):

11. The process of claim 10, wherein said site-directed **mutagenesis comprises mutagenesis** of the portion coding for **cysteine** at amino acid 500 to code for one selected from the group consisting of glycine, alanine, valine, leucine, isoleucine, serine, threonine, and **methionine**.

Claims Text - CLTX (17):

17. The process of claim 16, wherein said site-directed **mutagenesis comprises mutagenesis** of the portion coding for **cysteine** at amino acid 76 to code for one selected from the group consisting of glycine, alanine, valine, leucine, isoleucine, serine, threonine, and **methionine**.

US-PAT-NO: 5464747

DOCUMENT-IDENTIFIER: US 5464747 A

TITLE: Oxidation-resistant muteins of .beta.-galactosidase fragments

DATE-ISSUED: November 7, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Eisenbeis; Scott J.	Indianapolis	IN	N/A	N/A
Krevolin; Mark	Pinole	CA	N/A	N/A
Bryant; Christopher P.	Bourbonnais	IL	N/A	N/A
Boguslawski; Sophie J.	Indianapolis	IN	N/A	N/A
Ledden; David J.	Indianapolis	IN	N/A	N/A
Clark; Scott	Berkeley	CA	N/A	N/A

APPL-NO: 08/ 146633

DATE FILED: October 29, 1993

US-CL-CURRENT: 435/7.6, 435/18 , 435/188 , 435/207 , 435/69.1 , 435/963

ABSTRACT:

A mutein of an enzyme acceptor polypeptide fragment of .beta.-galactosidase which is resistant to oxidation is provided. The enzyme acceptor fragment has an amino acid other than cysteine located at position 602 of the natural sequence. An especially preferred substitution is serine. Also provided are a method for producing the novel mutein, a reagent composition comprising the novel mutein, and an immunoassay method for determining an analyte in which the novel mutein recombines with an enzyme donor polypeptide fragment to form enzymatically active .beta.-galactosidase.

16 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

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Brief Summary Text - BSTX (17):

Predetermined, site-directed **mutagenesis** of tRNA synthetase in which a **cysteine** residue is converted to serine has been reported (G. Winter et al., 1982, Nature, 299, 756-758, and A. Wilkinson et al., 1984, Nature, 307, 187-188). Estell et al., U.S. Pat. No. 4,760,025, issued Jul. 26, 1988

describe a cloned subtilisin gene modified at specific sites to cause amino acid substitutions of certain **methionine** residues. Koths et al., U.S. Pat. No. 4,752,585 issued Jun. 21, 1988 and U.S. Pat. No. 5,116,943, issued May 26, 1992, describe the protection of a therapeutic protein such as interleukin-2 or interferon-.beta. against oxidation by substituting a conservative amino acid for each methionyl residue susceptible to chloramine T or peroxide oxidation.

Brief Summary Text - BSTX (18):

Buchwalter et al., European Appl. No. 91106224.8, published Nov. 27, 1991, describe an animal somatotropin derivative in which **cysteine** residues are substituted by site-specific **mutagenesis** techniques for certain serine and tyrosine residues and in which glutamic acid has been substituted for certain **cysteine** residues. Breddam et al., PCT/DK91/00103 published Oct. 31, 1991, describe chemically modified detergent enzymes wherein one or more methionines have been **mutated** into cysteines, and then said cysteines are subsequently chemically modified in order to improve **stability of the enzyme toward oxidative** agents. Mattes et el., U.S. Pat. No. 4,963,469, issued Oct. 16, 1990, describe a change of an amino acid in the region between amino acid 430 and 550 of .beta.-galactosidase to another amino acid to produce an enzymatically inactive, immunologically active .beta.-galactosidase mutein. Estell et al. (1985, J. Biol. Chem. 260, 6518-6521) used site-directed **mutagenesis** to alter the **methionine** 222 residue of subtilisin which is a primary site for oxidative inactivation of the enzyme. These authors found that **mutants** containing non-oxidizable amino acids, i.e., serine, alanine and leucine, were resistant to peroxide inactivation, whereas **methionine and cysteine**-substituted enzymes were rapidly inactivated.

Brief Summary Text - BSTX (21):

The present invention provides novel muteins of enzyme acceptor polypeptide fragments of .beta.-galactosidase and processes for producing such muteins. In particular, the present invention provides novel muteins of enzyme acceptor polypeptide fragments of .beta.-galactosidase in which an amino acid other than cysteine is located at position 602. Particularly preferred are alpha-acceptor polypeptide fragments of .beta.-galactosidase in which serine is substituted for cysteine-602. Also provided are reagent compositions comprising these novel muteins and immunoassay methods utilizing such compositions in cloned enzyme donor immunoassays involving complementation between these enzymatically-inactive donor and acceptor fragments to form an enzymatically-active enzyme. The novel enzyme acceptor muteins have been found to exhibit substantially increased **stability and resistance to oxidation** over that of the parent enzyme acceptor fragment.

Detailed Description Text - DETX (2):

In accordance with the present invention, the novel enzyme acceptor polypeptide fragments of .beta.-galactosidase are prepared by site-directed **mutagenesis** methods, wherein a particular location on the gene coding for an enzyme acceptor fragment is **mutagenized**. In particular, site-directed **mutagenesis** methods are used to cause a **mutation** at the location coding for

cysteine at position 602 in the natural sequence, thereby causing the substitution of a conservative amino acid for **cysteine**. The preferred amino acid substitution is serine. Other amino acids may also be substituted, but conservative substitutions are preferred. By conservative substitution is meant replacement of **cysteine**-602 of .beta.-galactosidase by an amino acid which has similar characteristics and which is not likely to have an adverse effect on either the enzyme acceptor's ability to complement with enzyme donor or on the catalytic activity of the reformed .beta.-galactosidase. Examples of such conservative amino acid substitutions are glycine, alanine, valine, isoleucine, leucine, serine, threonine and **methionine**. An especially preferred substitution is serine, and an especially preferred parent enzyme acceptor is EA22, which is described fully in U.S. Pat. No. 4,708,929.

Claims Text - CLTX (6):

6. The process of claim 5, wherein said sitedirected **mutagenesis comprises mutagenesis** of the portion coding for **cysteine** at amino acid 602 to code for one selected from the group consisting of glycine, alanine, valine, isoleucine, leucine, serine, threonine, and **methionine**.

US-PAT-NO: 5455165

DOCUMENT-IDENTIFIER: US 5455165 A

TITLE: Expression vector encoding hybrid immunoglobulins

DATE-ISSUED: October 3, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Capon; Daniel J.	San Mateo	CA	N/A	N/A
Lasky; Laurence A.	Sausalito	CA	N/A	N/A

DISCLAIMER DATE: 20090526

APPL-NO: 08/ 185669

DATE FILED: January 21, 1994

PARENT-CASE:

This is a continuation of application Ser. No. 07/986,931 filed on 8 Dec. 1992, which is a continuation of application Ser. No. 07/808,122 filed 16 Dec. 1991, now U.S. Pat. No. 5,225,538, which is a division of application Ser. No. 07/440,624 filed 22 Nov. 1989, now U.S. Pat. No. 5,116,964, which is a continuation-in-part of application Ser. No. 07/315,015 filed 23 Feb. 1989, now U.S. Pat. No. 5,098,833.

US-CL-CURRENT: 435/69.7, 435/252.3 , 435/320.1 , 536/23.4

ABSTRACT:

Novel polypeptides are provided, together with methods for making and using them, and nucleic acids encoding them. These polypeptides are useful as cell surface adhesion molecules and ligands, and are useful in therapeutic or diagnostic compositions and methods.

11 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

---------- KWIC ---------

Detailed Description Text - DETX (77):

Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **xidative stability** of the LHR. Deletion or

substitutions of potential proteolysis sites, e.g. Arg Arg, is accomplished by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

Detailed Description Text - DETX (222):

Correct **mutants** were tested for expression by transfection onto human kidney 293 cells using previously described methods. 35S **methionine and cysteine** labeled supernatants were analyzed by immunoprecipitation with protein A sepharose beads in the absence of added antibodies. The precipitated proteins were analyzed on 7.5% polyacrylamide-SDS gels either with or without reduction with beta mercaptoethanol. Plasmids that resulted in correctly expressed chimeras were introduced into 293 cells by transfection in the presence of selective plasmids encoding resistance to G418 as well as dihydrofolate reductase. Clones were selected in G418, and the incorporated plasmids were amplified in the presence of methotrexate. Permanent cell lines expressing high levels of each construct were grown to large scale in T-flasks, and the cell supernatants were clarified by centrifugation and filtration. The resultant supernatants were concentrated by Amicon filtration and passed over standard protein A-sepharose columns, washed with PBS, and eluted with 0.1M Acetic Acid, 0.15M NaCl (pH 3.5). The eluted material was immediately neutralized with 3M Tris, pH 9, and quantitated by SDS gel electrophoresis as well as an ELISA assay.

US-PAT-NO: 5441882

DOCUMENT-IDENTIFIER: US 5441882 A

TITLE: Method for preparing modified subtilisins

DATE-ISSUED: August 15, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Estell; David A.	Mountain View	CA	N/A	N/A
Wells; James A.	San Mateo	CA	N/A	N/A
Bott; Richard R.	Burlingame	CA	N/A	N/A

APPL-NO: 07/ 521010

DATE FILED: May 9, 1990

PARENT-CASE:

This application is a continuation of U.S. patent application Ser. No. 07/091,235, filed Aug. 31, 1987 (now abandoned) which is a divisional application of U.S. patent application Ser. No. 06/614,612, filed May 29, 1984, issued as U.S. Pat. No. 4,760,025 on Jul. 26, 1988.

US-CL-CURRENT: 435/222, 435/252.31 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

There are described methods for making mutant subtilisins, the methods comprising obtaining a DNA fragment from a Bacillus subtilisin and introducing a mutation into the fragment by substituting at least one amino acid, transforming a suitable host cell with the mutated DNA, recovering a mutant subtilisin and screening the mutant subtilisin for certain altered enzymatic properties.

7 Claims, 25 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 23

---------- KWIC ---------

Brief Summary Text - BSTX (10):

Enzymes having characteristics which vary from available stock are required. In particular, enzymes having enhanced **oxidation stability** will be useful in extending the shelf life and bleach compatibility of proteases used in laundry

products. Similarly, reduced **xidati n stability** would be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity.

Brief Summary Text - BSTX (27):

Mutant enzymes are recovered which exhibit **oxidative stability** and/or pH-activity profiles which differ from the precursor enzymes. Procaryotic carbonyl hydrolases having varied Km, Kcat, Kcat/Km ratio and substrate specificity also are provided herein.

Drawing Description Text - DRTX (15):

FIG. 14 shows the enhanced **oxidation stability** of a subtilisin mutant.

Detailed Description Text - DETX (23):

The mutant carbonyl hydrolases expressed upon transformation of the suitable hosts are screened for enzymes exhibiting desired characteristics, e.g. substrate specificity, **oxidation stability,** pH-activity profiles and the like.

Detailed Description Text - DETX (26):

Oxidation stability is a further objective which is accomplished by **mutants** described in the examples. The stability may be enhanced or diminished as is desired for various uses. Enhanced stability is effected by deleting one or more methtonine, tryptophan, **cysteine** or lysine residues and, optionally, substituting another amino acid residue not one of **methionine,** tryptophan, **cysteine** or lysine. The opposite substitutions result in diminished **oxidation stability**. The substituted residue is preferably alanyl, but neutral residues al so are suitable.

Detailed Description Text - DETX (123):

B. subtilis strain BG2036 obtained by the method of Example 11 was transformed by the plasmids of Examples 14, 15 or 20 and by pS4-5 as a control. Transformants were plated or cultured in shaker flasks for 16 to 48 h at 37.degree. C. in LB media plus 12.5 .mu.g/ml chloramphenicol. Mutant enzymatically active subtilisin was recovered by dialyzing cell broth against 0.01M sodium phosphate buffer, pH 6.2. The dialyzed broth was then titrated to pH 6.2 with 1N HCl and loaded on a 2.5.times.2 cm column of CM cellulose (CM-52 Whatman). After washing with 0.01M sodium phosphate, pH 6.2, the subtilisins (except mutants at position +222) were eluted with the same buffer made 0.08N in NaCl. The mutant subtilisins at position +222 were each eluted with 0.1M sodium phosphate, pH 7.0. The purified mutant and wild type enzymes were then used in studies of **oxidation stability,** Km, Kcat, Kcat/Km ratio, pH optimum, and changes in substrate specificity.

Detailed Description Text - DETX (125):

Mutant Subtilisin Exhibiting Improved **Oxidation Stability**

Detailed Description Text - DETX (127):

To a total volume of 400 .mu.l of 0.1M, pH 7, NaPO.sub.4 buffer containing the indicated bleach concentrations (FIG. 14) sufficient enzyme was added to give a final concentration of 0.016 mg/ml of enzyme. The solutions were incubated at 25.degree. C. for 10 min. and assayed for enzyme activity as follows: 120 .mu.l of either ala+222 or wild type, or 100 .mu.l of the cys+222 incubation mixture was combined with 890 .mu.l 0.1M tris buffer at pH 8.6 and 10 .mu.l of a sAAPFpN (Example 18) substrate solution (20 mg/ml in DMSO). The rate of increase in absorbance at 410 nm due to release of p-nitroaniline (Del Mar, E. G., et al., 1979 "Anal. Biochem." 99, 316-320) was monitored. The results are shown in FIG. 14. The alanine substitution produced considerably more stable enzyme than either the wild-type enzyme or a **mutant** in which a labile **cysteine** residue was substituted for **methionine**. Surprisingly, the alanine substitution did not substantially interfere with enzyme activity against the assay substrate, yet conferred relative **oxidation stability** on the enzyme. The serine+222 **mutant** also exhibited improved **oxidation stability**.

Claims Text - CLTX (6):

e) screening said mutant subtilisin recovered in step d) for alterations of the enzyme characteristics of substrate specificity, **oxidative stability,** pH-activity profile, or the rate of formation of mature subtilisin from a subtilisin precursor.

Claims Text - CLTX (16):

f) screening said mutant subtilisin for alterations of the enzyme characteristics of substrate specificity, **oxidative stability,** pH-activity profile, or the rate of formation of mature subtilisin from a subtilisin precursor.

US-PAT-NO: 5428130

DOCUMENT-IDENTIFIER: US 5428130 A

TITLE: Hybrid immunoglobulins

DATE-ISSUED: June 27, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Capon; Daniel J.	San Mateo	CA	N/A	N/A
Lasky; Laurence A.	Sausalito	CA	N/A	N/A

DISCLAIMER DATE: 20090526

APPL-NO: 07/ 986931

DATE FILED: December 8, 1992

PARENT-CASE:

This application is a Continuation of application Ser. No. 07/808,122 filed on Dec. 16, 1991, now U.S. Pat. No. 5,225,538, which is a Continuation of application Ser. No. 07/440,625 filed Nov. 22, 1989, now U.S. Pat. No. 5,116,964, which is a Continuation-In-Part of U.S. Ser. No. 07/315,015, filed Feb. 23, 1989, now U.S. Pat. No. 5,098,833.

US-CL-CURRENT: 530/350, 435/69.7 , 530/387.1 , 536/23.4

ABSTRACT:

Novel polypeptides are provided, together with methods for making and using them, and nucleic acids encoding them. These polypeptides are useful as cell surface adhesion molecules and ligands, and are useful in therapeutic or diagnostic compositions and methods.

23 Claims, 19 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 14

---------- KWIC ---------

Detailed Description Text - DETX (78):

Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **oxidative stability** of the LHR. Deletion or substitutions of potential proteolysis sites, e.g. Arg Arg, is accomplished by

deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

Detailed Description Text - DETX (224):

Correct **mutants** were tested for expression by transfection onto human kidney 293 cells using previously described methods. 35S **methionine and cysteine** labeled supernatants were analyzed by immunoprecipitation with protein A sepharose beads in the absence of added antibodies. The precipitated proteins were analyzed on 7.5% polyacrylamide-SDS gels either with or without reduction with beta mercaptoethanol. Plasmids that resulted in correctly expressed chimeras were introduced into 293 cells by transfection in the presence of selective plasmids encoding resistance to G418 as well as dihydrofolate reductase. Clones were selected in G418, and the incorporated plasmids were amplified in the presence of methotrexate. Permanent cell lines expressing high levels of each construct were grown to large scale in T-flasks, and the cell supernatants were clarified by centrifugation and filtration. The resultant supernatants were concentrated by Amicon filtration and passed over standard protein A-sepharose columns, washed with PBS, and eluted with 0.1M Acetic Acid, 0.15M NaCl (pH 3.5). The eluted material was immediately neutralized with 3M Tris, pH 9, and quantitated by SDS gel electrophoresis as well as an ELISA assay.

US-PAT-NO: 5346823

DOCUMENT-IDENTIFIER: US 5346823 A

TITLE: Subtilisin modifications to enhance **oxidative stability**

DATE-ISSUED: September 13, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Estell; David A.	San Mateo	CA	N/A	N/A
Wells; James A.	Burlingame	CA	N/A	N/A
Bott; Richard R.	Burlingame	CA	N/A	N/A

DISCLAIMER DATE: 20110510

APPL-NO: 08/ 036592

DATE FILED: March 24, 1993

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 07/521,010 filed May 9, 1990 (pending), which is a continuation of U.S. patent application Ser. No. 07/091,235 filed Aug. 31, 1987 (abandoned), which is a divisional application of U.S. patent application Ser. No. 06/614,612 filed May 29, 1984, issued as U.S. Pat. No. 4,760,025.

US-CL-CURRENT: 435/222, 435/221 , 435/23 , 435/252.31 , 435/320.1 , 435/69.1 , 536/23.2

ABSTRACT:

There are provided methods for making a **mutant** Bacillus subtilisin having altered **oxidative stability,** the methods comprising obtaining DNA fragment consisting of a region coding for a Bacillus subtilisin, and introducing a **mutation** into said DNA fragment such that the **mutation** is introduced in a region encoding a **methionine,** tryptophan, **cysteine** or lysine, sensitive to oxidation, such that upon expression of the **mutant** subtilisin one or more codon regions encoding for **methionine,** tryptophan, **cysteine** or lysine is replaced with an amino acid other than **methionine,** tryptophan, **cysteine** or lysine, preferably alanine or serine.

4 Claims, 27 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 23

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Abstract Text - ABTX (1):
There are provided methods for making a **mutant** Bacillus subtilisin having altered **oxidative stability,** the methods comprising obtaining DNA fragment consisting of a region coding for a Bacillus subtilisin, and introducing a **mutation** into said DNA fragment such that the **mutation** is introduced in a region encoding a **methionine,** tryptophan, **cysteine** or lysine, sensitive to oxidation, such that upon expression of the **mutant** subtilisin one or more codon regions encoding for **methionine,** tryptophan, **cysteine** or lysine is replaced with an amino acid other than **methionine,** tryptophan, **cysteine** or lysine, preferably alanine or serine.

TITLE - TI (1):
Subtilisin modifications to enhance **oxidative stability**

Brief Summary Text - BSTX (10):
Enzymes having characteristics which vary from available stock are required. In particular, enzymes having enhanced **oxidation stability** will be useful in extending the shelf life and bleach compatibility of proteases used in laundry products. Similarly, reduced **oxidation stability** would be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity.

Brief Summary Text - BSTX (27):
Mutant enzymes are recovered which exhibit **oxidative stability** and/or pH-activity profiles which differ from the precursor enzymes. Procaryotic carbonyl hydrolases having varied Km, Kcat, Kcat/Km ratio and substrate specificity also are provided herein.

Drawing Description Text - DRTX (16):
FIG. 14 shows the enhanced **oxidation stability** of a subtilisin mutant.

Detailed Description Text - DETX (23):
The mutant carbonyl hydrolases expressed upon transformation of the suitable hosts are screened for enzymes exhibiting desired characteristics, e.g. substrate specificity, **oxidation stability,** pH-activity profiles and the like.

Detailed Description Text - DETX (26):
Oxidation stability is a further objective which is accomplished by mutants described in the examples. The stability may be enhanced or diminished as is desired for various uses. Enhanced stability is effected by deleting one or more methtonine, tryptophan, cysteine or lystne residues and, optionally, substituting another amino acid residue not one of methtonine, tryptophan,

cysteine or lysine. The opposite substitutions result in diminished **oxidation stability**. The substituted residue is preferably alanyl, but neutral residues also are suitable.

Detailed Description Text - DETX (123):

B. subtilis strain BG2036 obtained by the method of Example 11 was transformed by the plasmids of Examples 14, 1S or 20 and by pS4-5 as a control. Transformants were plated or cultured in shaker flasks for 16 to 48 h at 37.degree. C. in LB media plus 12.5 .mu.g/ml chloramphenicol. Mutant enzymatically active subtilisin was recovered by dialyzing cell broth against 0.01 M sodium phosphate buffer, pH 6.2. The dialyzed broth was then titrated to pH 6.2 with 1N HCl and loaded on a 2.5.times.2 cm column of CM cellulose (CM-52 Whatman). After washing with 0.01 M sodium phosphate, pH 6.2, the subtilisins (except mutants at position +222) were eluted with the same buffer made 0.08N in NaCl. The mutant subtilisins at position +222 were each eluted with 0.1 M sodium phosphate, pH 7.0. The purified mutant and wild type enzymes were then used in studies of **oxidation stability,** Kin, Kcat, Kcat/Km ratio, pH optimum, and changes in substrate specificity.

Detailed Description Text - DETX (125):

Mutant Subtilisin Exhibiting Improved **Oxidation Stability**

Detailed Description Text - DETX (127):

To a total volume of 400 .mu.l of 0.1 M, pH 7, NaPO.sub.4 buffer containing the indicated bleach concentrations (FIG. 14) sufficient enzyme was added to give a final concentration of 0.016 mg/ml of enzyme. The solutions were incubated at 25.degree. C. for 10 min. and assayed for enzyme activity as follows: 120 .mu.l of either ala+222 or wild type, or 100 .mu.l of the cys+222 incubation mixture was combined with 890 .mu.l M tris buffer at pH 8.6 and 10 .mu.l of a sAAPFpN (Example 18) substrate solution (20 mg/ml in DMSO). The rate of increase in absorbance at 410 nm due to release of p-nitroaniline (Del Mar, E.G., et al., 1979 "Anal. Biochem." 99, 316-320)was monitored. The results are shown in FIG. 14. The alanine substitution produced considerably more stable enzyme than either the wild-type enzyme or a **mutant** in which a labile **cysteine** residue was substituted for **methionine**. Surprisingly, the alanine substitution did not substantially interfere with enzyme activity against the assay substrate, yet conferred relative **oxidation stability** on the enzyme. The serine+222 **mutant** also exhibited improved **oxidation stability**.

Claims Text - CLTX (1):

1. A method for making a mutant Bacillus subtilisin having altered **oxidative stability,** the method comprising:

Claims Text - CLTX (7):

f) screening said mutant subtilisin for improved **oxidative stability** by assaying residual enzyme activity of the mutant in the presence of an oxidizing agent.

Claims Text - CLTX (8):

2. An isolated DNA segment encoding a **mutant** Bacillus subtilisin having altered **oxidative stability** wherein a codon encoding either serine of alanine is substituted for one or more codons selected from the group consisting of codons specifying **methionine,** tryptophan, **cysteine** or lysine, wherein said substituted codons are present in a DNA segment encoding a native or a modified subtilisin, and wherein said **methionine,** tryptophan, **cysteine** or lysine to be substituted is oxidized in the presence of an oxidizing agent.

US-PAT-NO: 5340735

DOCUMENT-IDENTIFIER: US 5340735 A

TITLE: Bacillus lentus alkaline protease variants with increased stability

DATE-ISSUED: August 23, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Christianson; Teresa	Cotati	CA	N/A	N/A
Goddette; Dean	Rohnert Park	CA	N/A	N/A
Ladin; Beth F.	Santa Rosa	CA	N/A	N/A
Lau; Maria R.	Fairfield	CA	N/A	N/A
Paech; Christian	Santa Rosa	CA	N/A	N/A
Reynolds; Robert B.	Santa Rosa	CA	N/A	N/A
Wilson; Charles R.	Santa Rosa	CA	N/A	N/A
Yang; Shiow-Shong	Santa Rosa	CA	N/A	N/A

APPL-NO: 07/ 706691

DATE FILED: May 29, 1991

US-CL-CURRENT: 435/221, 435/219 , 435/220 , 435/252.31 , 435/320.1 , 435/69.1 , 435/69.7 , 536/23.2 , 536/23.4 , 536/23.7

ABSTRACT:

Mutant B. lentus DSM 5483 proteases are derived by the replacement of at least one amino acid residue of the mature form of the B. lentus DSM 5483 alkaline protease. The mutant proteases are expressed by genes which are mutated by site-specific mutagenesis. The amino acid sites selected for replacement are identified by means of a computer based method which compares the three dimensional structure of the wild-type protease and a reference protease.

45 Claims, 18 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

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Brief Summary Text - BSTX (5):

Subtilisins are a family of extracellular proteins having molecular weights in the range of 25,000-35,000 daltons and are produced by various Bacillus

species. These proteins function as peptide hydrolases in that they catalyze the hydrolysis of peptide linkages in protein substrates at neutral and alkaline pH values. Subtilisins are termed serine proteases because they contain a specific serine residue which participates in the catalytic hydrolysis of peptide substrates. A subtilisin enzyme isolated from soil samples and produced by Bacillus lentus for use in detergent formulations having increased protease and **oxidative stability** over commercially available enzymes under conditions of pH 7 to 10 and at temperature of 10 to 60.degree. C. in aqueous solutions has been disclosed in copending patent application Ser. No. 07/398,854, filed on Aug. 25, 1989. This B. lentus alkaline protease enzyme (BLAP, vide infra) is obtained in commercial quantities by cultivating a Bacillus licheniformis ATCC 53926 strain which had been transformed by an expression plasmid which contained the wild type BLAP gene and the B. licheniformis ATCC 53926 alkaline protease gene promoter.

Brief Summary Text - BSTX (6):

Industrial processes generally are performed under physical conditions which require highly stable enzymes. Enzymes may be inactivated by high temperatures, pH extremes, oxidation, and surfactants. Even though Bacillus subtilisin proteases are currently used in many industrial applications, including detergent formulations, stability improvements are still needed. Market trends are toward more concentrated detergent powders, and an increase in liquid formulations. Increased shelf **stability and oxidative stability,** with retention of catalytic efficiency are needed. It is therefore desirable to isolate novel enzymes with increased stability, or to improve the stability of existing enzymes, including subtilisin proteases such as BLAP.

Brief Summary Text - BSTX (35):

EP 0251446 teaches the construction of mutant carbonyl hydrolases (proteases) which have at least one property different from the parental carbonyl hydrolase. It describes mutations which effect (either improve or decrease) **oxidative stability,** substrate specificity, catalytic activity, thermal stability, alkaline stability, pH activity profile, and resistance to autoproteolysis. These mutations were selected for introduction into Bacillus amyloliquefaciens subtilisin BPN' after alignment of the primary sequences of BPN' and proteases from B. subtilis, B. licheniformis, and thermitase. Such alignment can then be used to select amino acids in these other proteases which differ, as substitutes for the equivalent amino acid in the B. amyloliquefaciens carbonyl hydrolase. This application also describes alignment on the basis of a 1.8 .ANG. X-ray crystal structure of the B. amyloliquefaciens protease. Amino acids in the carbonyl hydrolase of B. amyloliquefaciens which when altered can affect stability, substrate specificity, or catalytic efficiency include: Met50, Met124, and Met222 for **oxidative stability**; Tyr104, Ala152, Glu156, Gly166, Gly169, Phe189, and Tyr217 for substrate specificity; N155 alterations were found to decrease turnover, and lower Km; Asp36, Ile 107, Lys170, Asp197, Ser204, Lys213, and Met222 for alkaline stability; and Met199, and Tyr21 for thermal stability. Alteration of other amino acids was found to affect multiple properties of the protease. Included in this category are Ser24, Met50, Asp156, Gly166, Gly169, and Tyr217. Substitution at residues Ser24, Met50, Ile107, Glu156, Gly166, Gly169, Ser204, Lys213, Gly215, and Tyr217 was predicted to increase thermal and alkaline

stability. An important point about this patent application is that with the exception of those mutations effecting substrate specificity, no rational mutational approach for improving the alkaline or temperature stability of a protease based upon computer simulations of an X-ray crystal structure is described.

Brief Summary Text - BSTX (39):

Sensitivity to oxidation is an important deficiency of serine proteases used in detergent applications (Stauffer, C.E., and Etson, D. (1969) J. Biol. Chemo 244:5333-5338). EP 0130756, EP 0247647, and U.S. Pat. No. 4,760,025 teach a saturation **mutation** method where one or multiple **mutations** are introduced into the subtilisin BPN' at amino acid residues Asp32, Asn155, Tyr104, Met222, Gly166, His64, Ser221, Gly169, Glu156, Ser33, Phe189, Tyr217, and/or Ala152. Using this approach **mutant** proteases exhibiting improved **oxidative stability,** altered substrate specificity, and/or altered pH activity profiles are obtained. A method is taught in which improved **oxidative stability** is achieved by substitution of **methionine, cysteine,** tryptophan, and lysine residues. These publications also teach that **mutations** within the active site region of the protease are also most likely to influence activity. Random or selected **mutations** can be introduced into a target gene using the experimental approach but neither EP 0130756, EP 0247647, nor U.S. Pat. No. 4,760,025 teach a method for predicting amino acid alterations which will improve the thermal or surfactant stability of the protease.

US-PAT-NO: RE34606

DOCUMENT-IDENTIFIER: US RE34606 E

TITLE: Modified enzymes and methods for making same

DATE-ISSUED: May 10, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Estell; David A.	San Mateo	CA	N/A	N/A
Wells; James A.	Burlingame	CA	N/A	N/A
Bott; Richard R.	Burlingame	CA	N/A	N/A

APPL-NO: 07/ 556918

DATE FILED: July 20, 1990

REISSUE-DATA:

US-PAT-NO	DATE-ISSUED	APPL-NO	DATE-FILED
04760025	July 26, 1988	614612	May 29, 1984

US-CL-CURRENT: 510/392, 435/221 , 435/222 , 510/530 , 930/200 , 930/240

ABSTRACT:

A cloned subtilsin gene has been modified at specific sites to cause amino acid substitutions at certain spots in the enzyme. The modified enzyme, preferably produced by Bacillus, is useful in combination with detergents.

20 Claims, 17 Drawing figures

Exemplary Claim Number: 4

Number of Drawing Sheets: 17

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Brief Summary Text - BSTX (12):

Enzymes having characteristics which vary from available stock are required. In particular, enzymes having enhanced **oxidation stability** will be useful in extending the shelf life and bleach compatibility of proteases used in laundry products. Similarly, reduced **oxidation stability** would be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity.

Brief Summary Text - BSTX (29):

Mutant enzymes are recovered which exhibit **oxidative stability** and/or

pH-activity profiles which differ from the precursor enzymes. Procaryotic carbonyl hydrolases having varied Km, Kcat, Kcat/Km ratio and substrate specificity also are provided herein.

Drawing Description Text - DRTX (16):
FIG. 14 shows the enhanced **oxidation stability** of a subtilisin mutant.

Detailed Description Text - DETX (24):
The mutant carbonyl hydrolases expressed upon transformation of the suitable hosts are screened for enzymes exhibiting desired characteristics, e.g. substrate specificity, **oxidation stability,** pH-activity profiles and the like.

Detailed Description Text - DETX (27):
Oxidation stability is a further objective which is accomplished by **mutants** described in the examples. The stability may be enhanced or diminished as is desired for various uses. Enhanced stability is effected by deleting one or more **methionine,** tryptophan, **cysteine** or lysine residues and, optionally, substituting another amino acid residue not one of **methionine,** tryptophan, **cysteine** or lysine. The opposite substitutions result in diminished **oxidation stability**. The substituted residue is preferably alanyl, but neutral residues also are suitable.

Detailed Description Text - DETX (124):
B. subtilis strain BG2036 obtained by the method of Example 11 was transformed by the plasmids of Examples 14, 15 or 20 and by pS4-5 as a control. Transformants were plated or cultured in shaker flasks for 16 to 48 h at 37.degree. C. in LB media plus 12.5 .mu.g/ml chloramphenicol. Mutant enzymatically active subtilisin was recovered by dialyzing cell broth against 0.01M sodium phosphate buffer, pH 6.2. The dialyzed broth was then titrated to pH 6.2 with 1N HCl and loaded on a 2.5.times.2 cm column of CM cellulose (CM-52 Whatman). After washing with 0.01M sodium phosphate, pH 6.2, the subtilisins (except mutants at position +222) were eluted with the same buffer made 0.08N in NaCl. The mutant subtilisins at position +222 were each eluted with 0.1M sodium phosphate, pH 7.0. The purified mutant and wild type enzymes were then used in studies of **oxidation stability,** Km, Kcat, Kcat/Km ratio, pH optimum, and changes in substrate specificity.

Detailed Description Text - DETX (126):
Mutant Subtilisin Exhibiting Improved **Oxidation Stability**

Detailed Description Text - DETX (128):
To a total volume of 400 .mu.l of 0.1M, pH 7, NaPO.sub.4 buffer containing the indicated bleach concentrations (FIG. 14) sufficient enzyme was added to give a final concentration of 0.016 mg/ml of enzyme. The solutions were incubated at 25.degree. C. for 10 min. and assayed for enzyme activity as follows: 120 .mu.l of either ala+222 or wild type, or 100 .mu.l of the cys+222

incubation mixture was combined with 890 .mu.l 0.1M tris buffer at pH 8.6 and 10 .mu.l of a sAAPFpN (Example 18) substrate solution (20 mg/ml in DMSO). The rate of increase in absorbance at 410 nm due to release of p-nitroaniline (Del Mar, E.G., et al., 1979 "Anal. Biochem." 99, 316-320) was monitored. The results are shown in FIG. 14. The alanine substitution produced considerably more stable enzyme than either the wild-type enzyme or a **mutant** in which a labile **cysteine** residue was substituted for **methionine**. Surprisingly, the alanine substitution did not substantially interfere with enzyme activity B against the assay substrate, yet conferred relative **oxidation stability** on the enzyme. The serine+222 **mutant** also exhibited improved **oxidation stability**.

Claims Text - CLTX (2):

17. .laddend. .ladd.10. A substantially pure modified subtilisin substituted at the residue position equivalent to ser+33 of the Bacillus amyloliquefaciens subtilisin shown in FIG. 1B with one of the other nineteen naturally occurring amino acids as shown in FIG. 17. .laddend. .ladd.11. A substantially pure modified subtilisin substituted at the residue position equivalent to gly+169 of the Bacillus amyloliquefaciens subtilisin shown in FIG. 1B with one of the other nineteen naturally occurring amino acids shown in FIG. 17. .laddend. .ladd.12. A substantially pure modified subtilisin substituted at the residue position equivalent to phe+189 of the Bacillus amyloliquefaciens subtilisin shown in FIG. 1B with one of the other nineteen naturally occurring amino acids shown in FIG. 17. .laddend. .ladd.13. A substantially pure modified subtilisin substituted at the residue position equivalent to tyr+217 of the Bacillus amyloliquefaciens subtilisin shown in FIG. 1B with one of the other nineteen naturally occurring amino acids shown in FIG. 17. .laddend. .ladd.14. A substantially pure modified subtilisin substituted at the residue position equivalent to glu+156 of the Bacillus amyloliquefaciens subtilisin shown in FIG. 1B with one of the other nineteen naturally occurring amino acids shown in FIG. 17. .laddend. .ladd.15. A substantially pure modified subtilisin substituted at the position equivalent to ala+152 of the Bacillus amyloliquefaciens subtilisin shown in FIG. 1B with one of the other nineteen naturally occurring amino acids shown in FIG. 17. .laddend. .ladd.16. A modified subtilisin according to claim 7 wherein said subtilisin has an improved pH activity profile when compared to said subtilisin having the amino acid naturally occurring in said subtilisin at the residue position equivalent to met+222. .laddend. .ladd.17. A modified subtilisin according to claim 8 wherein said subtilisin has improved substrate specificity when compared to said subtilisin having the amino acid naturally occurring in said subtilisin at the residue position equivalent to gly+166. .laddend. .ladd.18. A modified subtilisin according to claim 7 wherein said subtilisin has improved **oxidative stability** when compared to said subtilisin having the amino acid naturally occurring in said subtilisin at the residue position equivalent to met+222. .laddend. .ladd.19. A modified subtilisin according to claim 18 wherein the amino acid substituted at the residue position equivalent to met+222 is selected from the group consisting of ala, ser or cys. .laddend. .ladd.20. A substantially pure modified subtilisin having improved **oxidative stability** wherein the stability is effected by deleting one or more methionine, tryptophan, cysteine or lysine residue in said subtilisin and substituting another amino acid other than one of methionine, tryptophan, cysteine or lysine, for said deleted amino acid

US-PAT-NO: 5310675

DOCUMENT-IDENTIFIER: US 5310675 A

TITLE: Procaryotic carbonyl hydrolases

DATE-ISSUED: May 10, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Estell; David A.	Mountain View	CA	N/A	N/A
Ferrari; Eugenio	Daly City	CA	N/A	N/A
Henner; Dennis J.	Pacifica	CA	N/A	N/A
Wells; James A.	San Mateo	CA	N/A	N/A

APPL-NO: 07/ 805605

DATE FILED: December 10, 1991

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATION

This is a continuation, of application Ser. No. 352,326 filed May 15, 1989 now abandoned, which is a continuation of U.S. Ser. No. 866,389 filed on May 22, 1986, abandoned, which is a continuation of of U.S. Ser. No. 614,616, filed May 29, 1989 abandoned, which is a continuation-in-part of U.S. Ser. No. 507,419 filed Jun. 24, 1983, abandoned.

US-CL-CURRENT: 435/320.1, 435/219 , 435/221 , 435/222 , 435/252.3 , 435/252.5 , 536/23.1 , 536/23.2 , 536/23.7

ABSTRACT:

Recombinant host bacteria and plasmids for making the bacteria using recombinant DNA techniques are described. The plasmids contain DNA coding for subtilisin with an amino acid substitution. Expression of the plasmid DNA results in production of a modified subtilisin.

2 Claims, 27 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 23

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Brief Summary Text - BSTX (10):

Enzymes having characteristics which vary from available stock are required. In particular, enzymes having enhanced **oxidation stability** will be useful in extending the shelf life and bleach compatibility of proteases used in laundry products. Similarly, reduced **oxidation stability** would be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity.

Brief Summary Text - BSTX (27):

Mutant enzymes are recovered which exhibit **oxidative stability** and/or pH-activity profiles which differ from the precursor enzymes. Procaryotic carbonyl hydrolases having varied Km, Kcat, Kcat/Km ratio and substrate specificity also are provided herein.

Drawing Description Text - DRTX (16):

FIG.-14 shows the enhanced **oxidation stability** of a subtilisin mutant.

Detailed Description Text - DETX (24):

The mutant carbonyl hydrolases expressed upon transformation of the suitable hosts are screened for enzymes exhibiting desired characteristics, e.g. substrate specificity, **oxidation stability,** pH-activity profiles and the like.

Detailed Description Text - DETX (27):

Oxidation stability is a further objective which is accomplished by **mutants** described in the examples. The stability may be enhanced or diminished as is desired for various uses. Enhanced stability is effected by deleting one or more **methionine,** tryptophan, **cysteine** or lysine residues and, optionally, substituting another amino acid residue not one of **methionine,** tryptophan, **cysteine** or lysine. The opposite substitutions result in diminished **oxidation stability**. The substituted residue is preferably alanyl, but neutral residues also are suitable.

Detailed Description Text - DETX (123):

B. subtilis strain BG2036 obtained by the method of Example 11 was transformed by the plasmids of Examples 14, 15 or 20 and by pS4-5 as a control. Transformants were plated or cultured in shaker flasks for 16 to 48 h at 37.degree. C. in LB media plus 12.5 .mu.g/ml chloramphenicol. Mutant enzymatically active subtilisin was recovered by dialyzing cell broth against 0.01M sodium phosphate buffer, pH 6.2. The dialyzed broth was then titrated to pH 6.2 with 1N HCl and loaded on a 2.5.times.2 cm column of CM Cellulose (CM-52 Whatman). After washing with 0.01M sodium phosphate, pH 6.2, the subtilisins (except mutants at position +222) were eluted with the same buffer made 0.08N in NaCl. The mutant subtilisins at position +222 were each eluted with 0.1M sodium phosphate, pH 7.0. The purified mutant and wild type enzymes were then used in studies of **oxidation stability,** Km, Kcat, Kcat/Km ratio, pH optimum, and changes in substrate specificity.

Detailed Description Text - DETX (125):

Mutant Subtilisin Exhibiting Improved **Oxidation Stability**

Detailed Description Text - DETX (127):

To a total volume of 400 .mu.l of 0.01M, pH 7, NaPO.sub.4 buffer containing the indicated bleach concentrations (FIG. 14) sufficient enzyme was added to give a final concentration of 0.016 mg/ml of enzyme. The solutions were incubated at 25.degree. C. for 10 min. and assayed for enzyme activity as follows: 120 .mu.l of either ala+222 or wild type, or 100 .mu.l of the cys+222 incubation mixture was combined with 890 .mu.l 0.1M tris buffer at pH 8.6 and 10 .mu.l of a sAAPFpN (Example 18) substrate solution (20 mg/ml in DMSO). The rate of increase in absorbance at 410 nm due to release of p-nitroaniline (Del Mar, E. G., et al., 1979 "Anal. Biochem." 99, 316-210) was monitored. The results are shown in FIG. 14. The alanine substitution produced considerably more stable enzyme than either the wild-type enzyme or a **mutant** in which a labile **cysteine** residue was substituted for **methionine**. Surprisingly, the alanine substitution did not substantially interfere with enzyme activity against the assay substrate, yet conferred relative **oxidation stability** on the enzyme. The serine+222 **mutant** also exhibited improved **oxidation stability**.

US-PAT-NO: 5310661

DOCUMENT-IDENTIFIER: US 5310661 A

TITLE: Nucleic acid encoding the .alpha. chain prodomains of inhibin and method for synthesizing polypeptides using such nucleic acid

DATE-ISSUED: May 10, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mason; Anthony J.	San Francisco	CA	N/A	N/A
Seeburg; Peter H.	San Francisco	CA	N/A	N/A

APPL-NO: 07/ 958414

DATE FILED: October 8, 1992

PARENT-CASE:

This is a divisional application of U.S. Ser. No. 07/744,207 filed Aug. 12, 1991, now U.S. Pat. No. 5,215,893, which is a divisional application of U.S. Ser. No. 07/215,466 filed Jul. 5, 1988, now U.S. Pat. No. 5,089,396, which is a divisional application of U.S. Ser. No. 06/906,729 filed Dec. 31, 1986, now U.S. Pat. No. 4,798,885, which is a continuation-in-part application of U.S. Ser. No. 06/827,710 filed Feb. 7, 1986, now abandoned, which is a continuation-in-part application of U.S. Ser. No. 06/783,910 filed Oct. 3, 1985, now abandoned.

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/320.1 , 435/360 , 435/69.4 , 536/23.5 , 536/23.51

ABSTRACT:

DNA encoding the prepro inhibin .alpha. and .beta. chains has been isolated. This DNA is ligated into expression vectors and used to transform host cells for the preparation of inhibin or activin. Also provided are prohormone domains and other inhibin .alpha. or .beta. chain derivatives having therapeutic or diagnostic interest. The compositions provided herein are useful in the manipulation of fertility in animals.

16 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

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Detailed Description Text - DETX (16):

Insertional and deletional amino acid sequence variants are proteins in which one or more amino acid residues are introduced into or removed from a predetermined site in the target inhibin, activin, prodomain or proform of inhibin or activin. Most commonly, insertional variants are fusions of heterologous proteins or polypeptides to the amino or carboxyl terminus of the .alpha. or .beta. chains, the prodomains or other inhibin derivatives. Immunogenic derivatives are made by fusing an immunogenic polypeptide to the target sequence, e.g. a prodomain polypeptide, by synthesis in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Such immunogenic polypeptides preferably are bacterial polypeptides such as trpLE, beta-galactosidase and the like, together with their immunogenic fragments. Other insertions entail inserting heterologous eukaryotic (e.g. the herpes virus gD signal) or microbial secretion signal or protease processing sequences upstream from the NH.sub.2 -terminus of the protein to be secreted. Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **oxidative stability** of the .alpha. or .beta. chain. Deletional derivatives will produce .alpha. or .beta. chain fragments. Such fragments, when biologically or immunologically active, are within the scope herein. For instance, a fragment comprising .beta..sub.B or .beta..sub.A residues about from 11 to 45 (numbered from mature Gly.sub.1) is to be included within the scope herein.

Detailed Description Text - DETX (18):

Substitution derivatives are produced by **mutating** the DNA in a target codon, so that thereafter a different amino acid is encoded by the codon, with no concomitant change in the number of residues present in the molecule expressed from the **mutated** DNA. Substitutions or deletions are useful for example in increasing the stability of the proteins herein by eliminating proteolysis sites, wherein residues are substituted within or adjacent to the sites or are deleted from the sites, or by introducing additional disulfide bonds through the substitution of **cysteine** for other residues. Substitutions are useful for facilitating the synthesis or recovery of mature or prodomain .alpha. or .beta. chains. For example, **methionine** residues within the mature inhibin sequences are substituted or deleted, prepro sequences deleted, **methionine** is inserted at the -1 site immediately NH.sub.2 terminal to the mature NH.sub.2 terminal residue and another sequence inserted N-terminal to the exogenous **methionine**. The inhibin derivative in this case is expressed as a fusion having an intermediate methionyl residue, which in turn is cleaved at this residue by cyanogen bromide in accordance with known practice. The mature inhibin derivative released from the fusion is recovered.

US-PAT-NO: 5264366

DOCUMENT-IDENTIFIER: US 5264366 A

TITLE: Protease deficient bacillus

DATE-ISSUED: November 23, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ferrari; Eugenio	San Bruno	CA	N/A	N/A
Henner; Dennis J.	Pacifica	CA	N/A	N/A
Stahl; Mark L.	Arlington	MA	N/A	N/A

APPL-NO: 07/ 797577

DATE FILED: November 25, 1991

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation of application Ser. No. 041,885 filed Apr. 23, 1987, now abandoned, which is a continuation of application Ser. No. 614,615 filed May 29, 1984, now abandoned. Cross-reference is made to application Ser. No. 507,419 filed Jun. 24, 1983, and its continuation Ser. No. 614;616 filed May 29, 1984, abandoned in favor of Ser. No. 866,389 filed May 22, 1986. Cross reference is made to application Ser. No. 614,612 filed May 29, 1984, application Ser. No. 614,617 field May 29, 1984, and application Ser. No. 614,491 filed May 29, 1984, abandoned in favor of Ser. No. 924,162 filed Oct. 29, 1986.

US-CL-CURRENT: 435/252.31, 435/222 , 435/320.1 , 435/477 , 435/485 , 435/69.1

ABSTRACT:

There are described, normally sporulating mutant Bacillus strain(s) which produce no detectable proteolytic activity during any phase of its growth. The absence of detectable proteolytic activity is due to the deletion of one or more codons specifying the mature subtilisin protease and the mature neutral protease. Also described are methods for producing such normally sporulating, protease deficient Bacillus mutants.

4 Claims, 16 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 16

---------- KWIC ---------

Brief Summary Text - BSTX (10):
Enzymes having characteristics which vary from available stock are required. In particular, enzymes having enhanced **oxidation stability** will be useful in extending the shelf life and bleach compatibility of proteases used in laundry products. Similarly, reduced **oxidation stability** would be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity.

Brief Summary Text - BSTX (27):
Mutant enzymes are recovered which exhibit **oxidative stability** and/or ph-activity profiles which differ from the precursor enzymes. Procaryotic carbonyl hydrolases having varied Km, Kcat, Kcat/Km ratio and substrate specificity also are provided herein.

Drawing Description Text - DRTX (16):
FIG. 14 shows the enhanced **oxidation stability** of a subtilisin mutant.

Detailed Description Text - DETX (24):
The mutant carbonyl hydrolases expressed upon transformation of the suitable hosts are screened for enzymes exhibiting desired characteristics, e.g. substrate specificity, **oxidation stability,** pH-activity profiles and the like.

Detailed Description Text - DETX (27):
Oxidation stability is a further objective which is accomplished by **mutants** described in the examples. The stability may be enhanced or diminished as is desired for various uses. Enhanced stability is effected by deleting one or more **methionine,** tryptophan, **cysteine** or lysine residues and, optionally, substituting another amino acid residue not one of **methionine,** tryptophan, **cysteine** or lysine. The opposite substitutions result in diminished **oxidation stability**. The substituted residue is preferably alanyl, but neutral residues also are suitable.

Detailed Description Text - DETX (124):
B. subtilis strain BG2036 obtained by the method of Example 11 was transformed by the plasmids of Examples 14, 15 or 20 and by pS4-5 as a control. Transformants were plated or cultured in shaker flasks for 16 to 48 h at 37.degree. C. in LB media plus 12.5 .mu.g/ml chloramphenicol. Mutant enzymatically active subtilisin was recovered by dialyzing cell broth against 0.01M sodium phosphate buffer, pH 6.2. The dialyzed broth was then titrated to pH 6.2 with 1N HCl and loaded on a 2.5.times.2 cm column of CM cellulose (CM-52 Whatman). After washing with 0.01M sodium phosphate, pH 6.2, the subtilisins (except mutants at position +222) were eluted with the same buffer made 0.08N in NaCl. The mutant subtilisins at position +222 were each eluted with 0.1M sodium phosphate, pH 7.0. The purified mutant and wild type enzymes were then

used in studies of **xidati n stability,** Km, Kcat, Kcat/Km ratio, pH optimum, and changes in substrate specificity.

Detailed Description Text - DETX (126):
Mutant Subtilism Exhibiting Improved **Oxidation Stability**

Detailed Description Text - DETX (128):
To a total volume of 400 .mu.l of 0.1M, pH 7, NaPO.sub.4 buffer containing the indicated bleach concentrations (FIG. 14) sufficient enzyme was added to give a final concentration of 0.016 mg/ml of enzyme. The solutions were incubated at 25.degree. C. for 10 min. and assayed for enzyme activity as follows: 120 .mu.l of either ala+222 or wild type, or 100 .mu.l of the cys+222 incubation mixture was combined with 890 .mu.l 0.1M tris buffer at pH 8.6 and 10 .mu.l of a sAAPFpN (Example 18) substrate solution (20 mg/ml in DMSO). The rate of increase in absorbance at 410 nm due to release of p-nitroaniline (Del Mar, E. G., et al., 1979 "Anal. Biochem.",99, 316-320) was monitored. The results are shown in FIG. 14. The alanine substitution produced considerably more stable enzyme than either the wild-type enzyme or a **mutant** in which a labile **cysteine** residue was substituted for **methionine**. Surprisingly, the alanine substitution did not substantially interfere with enzyme activity against the assay substrate, yet conferred relative **oxidation stability** on the enzyme. The serine+222 **mutant** also exhibited improved **oxidation stability**.

US-PAT-NO: 5225538

DOCUMENT-IDENTIFIER: US 5225538 A

TITLE: Lymphocyte homing receptor/immunoglobulin fusion proteins

DATE-ISSUED: July 6, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Capon; Daniel J.	San Mateo	CA	N/A	N/A
Lasky; Laurence A.	Sausalito	CA	N/A	N/A

APPL-NO: 07/ 808122

DATE FILED: December 16, 1991

PARENT-CASE:

This is a continuation of co-pending applicaton Ser. No. 07/440,625 filed on Nov. 22, 1989, now U.S. Pat. No. 5,116,947, which is a continuation-in-part of application Ser. No. 07/315,015, filed on Feb. 23, 1989, now U.S. Pat. No. 5,098,833.

US-CL-CURRENT: 530/387.3, 424/134.1 , 435/69.7 , 530/388.73

ABSTRACT:

Novel polypeptides are provided, together with methods for making and using them, and nucleic acids encoding them. These polypeptides are useful as cell surface adhesion molecules and ligands, and are useful in therapeutic or diagnostic compositions and methods.

29 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

---------- KWIC ---------

Detailed Description Text - DETX (78):

Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **oxidative stability** of the LHR. Deletion or substitutions of potential proteolysis sites, e.g. Arg Arg, is accomplished by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

Detailed Description Text - DETX (219):

Correct **mutants** were tested for expression by transfection onto human kidney 293 cells using previously described methods. 35S **methionine and cysteine** labeled supernatants were analyzed by immunoprecipitation with protein A sepharose beads in the absence of added antibodies. The precipitated proteins were analyzed on 7.5% polyacrylamide-SDS gels either with or without reduction with beta mercaptoethanol. Plasmids that resulted in correctly expressed chimeras were introduced into 293 cells by transfection in the presence of selective plasmids encoding resistance to G418 as well as dihydrofolate reductase. Clones were selected in G418, and the incorporated plasmids were amplified in the presence of methotrexate. Permanent cell lines expressing high levels of each construct were grown to large scale in T-flasks, and the cell supernatants were clarified by centrifugation and filtration. The resultant supernatants were concentrated by Amicon filtration and passed over standard protein A-sepharose columns, washed with PBS, and eluted with 0.1M Acetic Acid, 0.15M NaCl (pH 3.5). The eluted material was immediately neutralized with 3M Tris, pH 9, and quantitated by SDS gel electrophoresis as well as an ELISA assay.

US-PAT-NO: 5215893

DOCUMENT-IDENTIFIER: US 5215893 A

TITLE: Nucleic acid encoding the ba chain prodomains of inhibin and method for synthesizing polypeptides using such nucleic acid

DATE-ISSUED: June 1, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mason; Anthony J.	San Francisco	CA	N/A	N/A
Seeburg; Peter H.	Heidelberg	N/A	N/A	DE

APPL-NO: 07/ 744207

DATE FILED: August 12, 1991

PARENT-CASE:

This is a divisional application of copending U.S. Ser. No. 07/215,466, filed Jul. 5, 1988, U.S. Pat. No. 5,089,396, which is a divisional application of U.S. Ser. No. 906,729, filed Dec. 31, 1986, U.S. Pat. No. 4,798,885 which is a continuation-in-part application of U.S. Ser. No. 827,710, filed Feb. 7, 1986, now abandoned, which is in turn a continuation-in-part application of U.S. Ser. No. 783,910, filed Oct. 3, 1985, now abandoned.

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/320.1 , 435/360 , 435/69.4 , 536/23.5 , 536/23.51

ABSTRACT:

DNA encoding the prepro inhibin .alpha. and .beta. chains has been isolated. This DNA is ligated into expression vectors and used to transform host cells for the preparation of inhibin or activin. Also provided are prohormone domains and other inhibin .alpha. and .beta. chain derivatives having therapeutic or diagnostic interest. The compositions provided herein are useful in the manipulation of fertility in animals.

17 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

---------- KWIC ---------

Detailed Description Text - DETX (16):

Insertional and deletional amino acid sequence variants are proteins in which one or more amino acid residues are introduced into or removed from a predetermined site in the target inhibin, activin, prodomain or proform of inhibin or activin. Most commonly, insertional variants are fusions of heterologous proteins or polypeptides to the amino or carboxyl terminus of the .alpha. or .beta. chains, the prodomains or other inhibin derivatives. Immunogenic derivatives are made by fusing an immunogenic polypeptide to the target sequence, e.g. a prodomain polypeptide, by synthesis in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Such immunogenic polypeptides preferably are bacterial polypeptides such as trpLE, betagalactosidase and the like, together with their immunogenic fragments. Other insertions entail inserting heterologous eukaryotic (e.g. the herpes virus gD signal) or microbial secretion signal or protease processing sequences upstream from the NH.sub.2 -terminus of the protein to be secreted. Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **oxidative stability** of the .alpha. or .beta. chain. Deletional derivatives will produce .alpha. or .beta. chain fragments. Such fragments, when biologically or immunologically active, are within the scope herein. For instance, a fragment comprising .beta..sub.B or .beta..sub.A residues about from 11 to 45 (numbered from mature Gly.sub.1) is to be included within the scope herein.

Detailed Description Text - DETX (18):

Substitution derivatives are produced by **mutating** the DNA in a target codon, so that thereafter a different amino acid is encoded by the codon, with no concomitant change in the number of residues present in the molecule expressed from the **mutated** DNA. Substitutions or deletions are useful for example in increasing the stability of the proteins herein by eliminating proteolysis sites, wherein residues are substituted within or adjacent to the sites or are deleted from the sites, or by introducing additional disulfide bonds through the substitution of **cysteine** for other residues. Substitutions are useful for facilitating the synthesis or recovery of mature or prodomain .alpha. or .beta. chains. For example, **methionine** residues within the mature inhibin sequences are substituted or deleted, prepro sequences deleted, **methionine** is inserted at the -1 site immediately NH.sub.2 terminal to the mature NH.sub.2 terminal residue and another sequence inserted N-terminal to the exogenous **methionine**. The inhibin derivative in this case is expressed as a fusion having an intermediate methionyl residue, which in turn is cleaved at this residue by cyanogen bromide in accordance with known practice. The mature inhibin derivative released from the fusion is recovered.

US-PAT-NO: 5208158

DOCUMENT-IDENTIFIER: US 5208158 A

TITLE: **Oxidation stable** detergent enzymes

DATE-ISSUED: May 4, 1993

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bech; Lene M.	Naestved	N/A	N/A	DK
Branner; Sven	Lyngby	N/A	N/A	DK
Breddam; Klaus	Glostrup	N/A	N/A	DK
Groen; Hanne	Copenhagan	N/A	N/A	DK

APPL-NO: 07/ 553934

DATE FILED: July 17, 1990

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DK	971/90	April 19, 1990

US-CL-CURRENT: 435/219, 435/183 , 435/188 , 435/198 , 435/201 , 435/209

ABSTRACT:

Novel chemically modified detergent enzymes are provided, wherein one or more methionines have been mutated into cysteines, said cysteines subsequently being chemically modified in order to confer the enzyme improved **stability towards oxidative** agents. A novel process for **stabilizing detergent enzymes against oxidation** is also provided. Furthermore, there are provided detergent compositions comprising these novel **oxidation stable** detergent enzymes.

12 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

---------- KWIC ---------

Abstract Text - ABTX (1):

Novel chemically modified detergent enzymes are provided, wherein one or more methionines have been mutated into cysteines, said cysteines subsequently being chemically modified in order to confer the enzyme improved **stability t wards oxidative** agents. A novel process for **stabilizing detergent enzymes against oxidation** is also provided. Furthermore, there are provided detergent

compositions comprising these novel **oxidati_n stable** detergent enzymes.

TITLE - TI (1):
Oxidati_n stable detergent enzymes

Brief Summary Text - BSTX (2):
This invention is within the field of **oxidation stable** detergent enzymes. More specifically, the present invention relates to novel chemically modified detergent enzymes wherein one or more methionines have been mutated into cysteines, said cysteines subsequently being chemically modified in order to confer the enzyme improved **stability towards oxidative** agents. The present invention is also directed towards a novel process for **stabilizing detergent enzymes against oxidation**. Further, the present invention is directed towards a detergent composition comprising these novel **oxidation stable** detergent enzymes.

Brief Summary Text - BSTX (4):
The problems related to a generally low **oxidation stability** is a well known major obstacle in respect to the activity of detergent enzymes. Due to the presence of bleach active ingredients, the detergent enzymes have to perform their enzymatic action in an oxidative environment, with a consequent loss of activity.

Brief Summary Text - BSTX (5):
Various solutions have been proposed to the problem, but hitherto **oxidation stable** detergent enzymes have not been available.

Brief Summary Text - BSTX (9):
Substitution of the residue at position 222 with any other of the 19 essential amino acid residues has been carried out, and the mutants obtained were investigated in respect to relative activity (J. Biol. Chem., 260, 6518-6521 (1985)). Only the Cys-mutant displayed a relative activity in magnitude of the wildtype (56%), but similar to the wildtype this Cys-mutant was unstable to oxidative agents, and a "half life" in the order of 12 minutes in 1 M H.sub.2 O.sub.2 was found (the wildtype-subtilisins do not contain cystein residues). Among the most **oxidation stable** mutants the Ala-mutant displayed the highest enzymatic activity (11%), and it did not lose its activity even after 15 minutes in 1 M H.sub.2 O.sub.2.

Brief Summary Text - BSTX (11):
Therefore, it is an object of the present invention to provide novel chemically altered detergent enzymes, exhibiting improved oxidation resistance, and at the same time substantially retaining their proteolytic activity in respect of their wash performance. Further, it is an object of the present invention to establish a novel process for **stabilizing detergent enzymes against oxidation**.

Brief Summary Text - BSTX (13):
By way of chemical modification of variants of detergent enzymes, the preparation of novel active chemically modified detergent enzymes, with conferred **stability towards oxidative** agents, has now surprisingly succeeded.

Brief Summary Text - BSTX (14):
Thus, according to a first aspect, the present invention provides novel **oxidation stable** chemically modified detergent enzymes, wherein one or more methionines have been mutated into cysteines, and the cysteines are subsequently modified chemically in order to substitute the hydrogen of the HS-group into a group of the general formula R.sup.1 S--, wherein R.sup.1 is C.sub.1-6 -alkyl.

Brief Summary Text - BSTX (15):
According to a second aspect, the present invention provides a novel process for **stabilizing detergent enzymes against oxidation,** whereby a variant of the detergent enzyme, wherein, one or more methionines have been mutated into cysteine, are chemically modified by substitution of the hydrogen of the HS-group of the cysteines into a group of the general formula R.sup.1 S--, wherein R.sup.1 is C.sub.1-6 -alkyl, due to reaction with a compound of the general formula R.sup.1 SSO.sub.2 R.sup.2, wherein R.sup.1 is C.sub.1-6 -alkyl and R.sup.2 is C.sub.1-4 -alkyl, and the reaction is carried out at pH values in the range 5 to 11.

Brief Summary Text - BSTX (16):
According to a third aspect, the present invention provides a detergent composition comprising one or more of the **oxidation stable** detergent enzymes, provided in the form of a detergent additive, preferably a non-dusting granulate or a stabilized liquid.

Drawing Description Text - DRTX (6):
FIG. 2b shows an enzyme variant, wherein a **methionine has been mutated into a cysteine** (M.fwdarw.C).

Detailed Description Text - DETX (2):
By a chemical modification process according to the present, invention, detergent enzymes, wherein one or more methionines have been **mutated** into cysteines, are treated with an agent of the general formula R.sup.1 SSO.sub.2 R.sup.2, wherein R.sup.1 and R.sup.2 are defined below, in order to substitute the hydrogen of the HS-group of the **cysteine** into a group of the general formula R.sup.1 S--(cf. FIG. 1). By this process, **cysteine** is changed into an amino acid that sterically resembles the **methionine** originally present, but is much more **stable towards oxidative** agents than **methionine** (cf. FIG. 2), and an **xidation stable** detergent enzyme of the invention is obtained.

Detailed Description Text - DETX (6):
By the process for **stabilizing detergent nzymes against xidation** of the invention, a variant of the detergent enzyme, wherein one or been methionines have been mutated into cysteines, are chemically modified by substitution of the HS-group of the cysteines into a group of the general formula R.sup.1 SS--, wherein R.sup.1 is C.sub.1-6 -alkyl, due to reaction with a compound of the general formula R.sup.1 SSO.sub.2 R.sup.2, wherein R.sup.1 is C.sub.1-6 -alkyl, and R.sup.2 is C.sub.1-4 -alkyl.

Detailed Description Text - DETX (8):
In the process for **stabilizing detergent enzymes against oxidation** of the invention, starting compounds are variants of detergent enzymes, wherein one or more methionines have been mutated into cysteines.

Detailed Description Text - DETX (19):
The novel **oxidation stable** chemically modified detergent enzymes of the invention are detergent enzymes, wherein one or more methionines have been mutated, into cysteines, followed by chemically modification of the cysteines, in order to substitute the hydrogen of the HS-group into a group of the general formula R.sup.1 S--, wherein R.sup.1 is C.sub.1-6 -alkyl.

Detailed Description Text - DETX (20):
Preferred **oxidation stable** detergent enzymes of the invention are amylases, lipases, cellulases or proteases wherein one or more methionines have been mutated into cysteines in order to substitute the hydrogen of the HS-group into a group of the general formula R.sup.1 S--, wherein R.sup.1 is C.sub.1-6 -alkyl.

Detailed Description Text - DETX (21):
More preferred **oxidation stable** detergent enzymes of the invention are subtilisins.

Detailed Description Text - DETX (22):
Most preferred **oxidation stable** detergent enzymes of the invention are amylases, lipases, cellulases or proteases, wherein one or more methionines have been mutated into cysteines in order to substitute the hydrogen of the HS-group into a group of the general formula R.sup.1 S--, wherein R.sup.1 is C.sub.1-3 -alkyl.

Detailed Description Text - DETX (23):
In another embodiment of the invention, preferred **oxidation stable** detergent enzymes are chemically modified subtilisins, wherein the **methionine** in position 222, adjacent to the active serine, has been **mutated into cysteine,** followed by chemical modification, in order to substitute the hydrogen of the HS-group of the cysteines into a group of the general formula R.sup.1 S--, wherein R.sup.1

is C.sub.1-6 -alkyl.

Detailed Description Text - DETX (24):
More preferred are **oxidati n stable** chemically modified subtilisins, where the subtilisins are subtilisin-309, subtilisin-147, subtilisin BPN', subtilisin Carlsberg or proteinase K.

Detailed Description Text - DETX (25):
Most preferred is a **oxidation stable** chemically modified subtilisin, which is subtilisin-309.

Detailed Description Text - DETX (27):
According to the invention, any detergent enzyme can be modified chemically in order to achieve an improved **oxidation stability**. Preferred **oxidation stable** chemically modified detergent enzymes according to the invention are subtilisin-309-M222C-SM and subtilisin-309-M222C-SP.

Detailed Description Text - DETX (29):
As appears from the table, K.sub.M is nearly constant for all enzymes, whereas k.sub.cat varies considerably. The "wild type" enzyme possesses the highest specific activity, but evidently, the subtilisin-309-M222C-SM enzyme possesses the best **stability towards oxidative** environments along with relatively high enzymatic activity.

Detailed Description Text - DETX (37):
Due to a novel chemical modification process according to the present invention it is therefore possible to obtain novel subtilisin variants. These novel chemically modified subtilisin variants according to the present invention possess excellent **stability towards oxidative** agents as well as a relatively high enzymatic activity. Moreover, their wash performance is retained in comparison to their parent enzymes.

US-PAT-NO: 5118623

DOCUMENT-IDENTIFIER: US 5118623 A
See image for Certificate of Correction

TITLE: Bleach stable enzymes

DATE-ISSUED: June 2, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Boguslawski; George	South Bend	IN	N/A	N/A
Shultz; John W.	Elkhart	IN	N/A	N/A

APPL-NO: 07/ 199511

DATE FILED: May 27, 1988

US-CL-CURRENT: 510/374, 435/220 , 435/221 , 435/222 , 510/226 , 510/306 , 510/320 , 510/392 , 510/530

ABSTRACT:

The methods of the invention can be used to identify sites of cleavage of an enzyme in the presence of hypochlorite. It has been found that such cleavage occurs at tryptophan. Chemical modifications or genetic manipulation to change or delete tryptophan can be done to produce a more stable enzyme which retains activity in the presence of hypochlorite. The invention is particularly applicable to alkaline proteases which are useful in detergent compositions.

6 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

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Brief Summary Text - BSTX (9):

Suggestions have been made to improve the properties of alkaline proteases by site directed **mutagenesis**. In European Patent Application 0130756 published Jan. 9, 1985, a method for preparing an improved carbonyl hydrolase by site directed **mutagenesis** was disclosed. The application suggests that **oxidative stability,** pH activity profiles, K.sub.m, k.sub.cat, k.sub.cat /K.sub.m ratios and substrate specificity can be improved by the substitution, deletion or insertion of at least one amino acid at a predetermined site in the hydrolase. Specifically it was suggested that enhanced **xidative stability** can be effected by deleting one or more **methionine,** tryptophan, **cysteine** or lysine residues

and, optionally, substituting another amino acid residue not one of **methi nine,** tryptophan, **cysteine** or lysine, preferably alanine but alternatively neutral residues.

Brief Summary Text - BSTX (10):

However, alkaline proteases contain from about 250 to about 290 amino acids and may contain 15 to 18 sites which fit the description above. It would require an inordinate amount of experimentation to perform site directed mutagenesis at each of those sites and to express and test each substitution for improved **oxidative stability**.

Drawing Description Text - DRTX (25):

While it is known that enzymes, in particular alkaline proteases, are inactivated in the presence of hypochlorite, it is generally believed that such inactivation is due to oxidation of amino acids such as methionine (Stauffer, C. E. et al., 1969, J. Biol. Chem., 244, 5333-5338). Therefore previous work has focused on the possible oxidation of methionine. Methionine is oxidized to methionine sulfoxide or methionine sulfone but the peptide backbone is not cleaved. EP 0130756, while suggesting that replacement or deletion of one or more methionine, tryptophan, cysteine or lysine might improve **oxidative stability,** disclosed examples of replacement of methionine to improve **oxidative stability**.

US-PAT-NO: 5116964

DOCUMENT-IDENTIFIER: US 5116964 A

TITLE: Hybrid immunoglobulins

DATE-ISSUED: May 26, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Capon; Daniel J.	San Mateo	CA	N/A	N/A
Lasky; Laurence A.	Sausalito	CA	N/A	N/A

APPL-NO: 07/ 440625

DATE FILED: November 22, 1989

PARENT-CASE:

This is a continuation-in-part of U.S. Ser. No. 07/315,015, filed Feb. 23, 1989.

US-CL-CURRENT: 536/23.5, 424/134.1 , 435/252.3 , 435/320.1 , 435/69.7 , 530/350 , 530/387.3 , 536/23.51 , 536/23.53

ABSTRACT:

Immunoglobulin fusion polypeptides are provided, together with methods for making and using them, and nucleic acids encoding them. These polypeptides are useful as cell surface adhesion molecules and ligands, and are useful in therapeutic or diagnostic compositions and methods.

8 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 18

---------- KWIC ---------

Detailed Description Text - DETX (57):

Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **oxidative stability** of the LHR. Deletion or substitutions of potential proteolysis sites, e.g. Arg Arg, is accomplished by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

Detailed Description Text - DETX (202):

Correct **mutants** were tested for expression by transfection onto human kidney 293 cells using previously described methods. 35S **methionine and cysteine** labeled supernatants were analyzed by immunoprecipitation with protein A sepharose beads in the absence of added antibodies. The precipitated proteins were analyzed on 7.5% polyacrylamide-SDS gels either with or without reduction with beta mercaptoethanol. Plasmids that resulted in correctly expressed chimeras were introduced into 293 cells by transfection in the presence of selective plasmids encoding resistance to G418 as well as dihydrofolate reductase. Clones were selected in G418, and the incorporated plasmids were amplified in the presence of methotrexate. Permanent cell lines expressing high levels of each construct were grown to large scale in T-flasks, and the cell supernatants were clarified by centrifugation and filtration. The resultant supernatants were concentrated by Amicon filtration and passed over standard protein A-sepharose columns, washed with PBS, and eluted with 0.1M Acetic Acid, 0.15M NaCl (pH 3.5). The eluted material was immediately neutralized with 3M Tris, pH 9. and quantitated by SDS gel electrophoresis as well as an ELISA assay.

US-PAT-NO: 5089396

DOCUMENT-IDENTIFIER: US 5089396 A

TITLE: Nucleic acid encoding .beta. chain prodomains of inhibin and method for synthesizing polypeptides using such nucleic acid

DATE-ISSUED: February 18, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mason; Anthony J.	San Francisco	CA	N/A	N/A
Seeburg; Peter H.	San Francisco	CA	N/A	N/A

APPL-NO: 07/ 215466

DATE FILED: July 5, 1988

PARENT-CASE:

This is a divisional application of 5/7/91 U.S. Ser. No. 906,729, filed Dec. 31, 1986, now U.S. Pat. No. 4,798,885, which is a continuation-in-part application of U.S. Ser. No. 827,710, filed Feb. 7, 1986, now abandoned, which is in turn a continuation-in-part application of U.S. Ser. No. 783,910, filed Oct. 3, 1985, now abandoned.

US-CL-CURRENT: 435/69.1, 435/252.3 , 435/320.1 , 435/360 , 435/69.4 , 536/23.51

ABSTRACT:

DNA encoding the prepro inhibin .alpha. .and .beta. chains has been isolated. This DNA is ligated into expression vectors and used to transform host cells for the preparation of inhibin or activin. Also provided are prohormone domains and other inhibin .alpha. and .beta. chain derivatives having therapeutic or diagnostic interest. The compositions provided herein are useful in the manipulation of fertility in animals.

16 Claims, 20 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

---------- KWIC ---------

Detailed Description Text - DETX (16):

Insertional and deletional amino acid sequence variants are proteins in which one or more amino acid residues are introduced into or removed from a predetermined site in the target inhibin, activin, prodomain or proform of inhibin or activin. Most commonly, insertional variants are fusions of heterologous proteins or polypeptides to the amino or carboxyl terminus of the .alpha. or .beta. chains, the prodomains or other inhibin derivatives. Immunogenic derivatives are made by fusing an immunogenic polypeptide to the target sequence, e.g. a prodomain polypeptide, by synthesis in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Such immunogenic polypeptides preferably are bacterial polypeptides such as trpLE, beta-galactosidase and the like, together with their immunogenic fragments. Other insertions entail inserting heterologous eukaryotic (e.g. the herpes virus gD signal) or microbial secretion signal or protease processing sequences upstream from the NH.sub.2 -terminus of the protein to be secreted. Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **oxidative stability** of the .alpha. or .beta. chain. Deletional derivatives will produce .alpha. or .beta. chain fragments. Such fragments, when biologically or immunologically active, are within the scope herein. For instance, a fragment comprising .beta..sub.B or .beta..sub.A residues about from 11 to 45 (numbered from mature Gly.sub.1) is to be included within the scope herein.

Detailed Description Text - DETX (18):

Substitution derivatives are produced by **mutating** the DNA in a target codon, so that thereafter a different amino acid is encoded by the codon, with no concomitant change in the number of residues present in the molecule expressed from the **mutated** DNA. Substitutions or deletions are useful for example in increasing the stability of the proteins herein by eliminating proteolysis sites, wherein residues are substituted within or adjacent to the sites or are deleted from the sites, or by introducing additional disulfide bonds through the substitution of **cysteine** for other residues. Substitutions are useful for facilitating the synthesis or recovery of mature or prodomain .alpha. or .beta. chains. For example, **methionine** residues within the mature inhibin sequences are substituted or deleted, prepro sequences deleted, **methionine** is inserted at the -1 site immediately NH.sub.2 terminal to the mature NH.sub.2 terminal residue and another sequence inserted N-terminal to the exogenous **methionine**. The inhibin derivative in this case is expressed as a fusion having an intermediate methionyl residue, which in turn is cleaved at this residue by cyanogen bromide in accordance with known practice. The mature inhibin derivative released from the fusion is recovered.

US-PAT-NO: 4904602

DOCUMENT-IDENTIFIER: US 4904602 A
See image for Certificate of Correction

TITLE: Thioredoxin shufflease and use thereof

DATE-ISSUED: February 27, 1990

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Pigiet; Vincent P.	Winchester	MA	N/A	N/A
Rusche; James R.	Worcester	MA	N/A	N/A
Schuster; Barbara J.	State College	PA	N/A	N/A

APPL-NO: 06/ 894421

DATE FILED: August 8, 1986

PARENT-CASE:

CROSS REFERENCE TO A RELATED APPLICATION

This is a continuation-in-part of our copending application Ser. No. 802,569, filed on Nov. 27, 1985, now abandoned.

US-CL-CURRENT: 435/191, 435/189

ABSTRACT:

The subject invention concerns a novel enzyme named thioredoxin shufflease, means for preparing the same, and procedures for using thioredoxin shufflease to fold proteins containing disulfide crosslinks. Thioredoxin shufflease is a generic term to define enzymes which have the following characteristics: (a) contain a single reactive thiol group; (b) catalyze the exchange of disulfides in a protein undergoing the refolding process; and (c) are not consumed in the oxidation/refolding process. Specifically exemplified is a thioredoxin shufflease produced from an E. coli thioredoxin gene.

10 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (2):

When proteins that contain disulfide bonds are produced in microorganisms they are made in a reduced form that lacks the critical and correct disulfide

bonds required for activity. On cell breakage, these proteins are often insoluble because of their non-native conformations and have non-native incorrect disulfide pairing. Various approaches have been used to correctly fold these proteins, which generally require reduction of all disulfides followed by carrying out a controlled oxidation reaction. This controlled oxidation reaction must provide a suitable oxidation environment to oxidatively transform protein thiols into disulfides as well as to allow for the necessary exchange reactions. Conditions that promote effective disulfide interchange require a balance between **oxidation and reduction such that the greater thermodynamic stability** of the native, or correct, disulfides will be the driving force for achieving the native structure. A consequence of this necessary balance between the need for oxidative drive for disulfide formation and a suitable redox environment for disulfide exchange is that either too high or too low an oxidation environment will compromise the yield of native protein. Examples of incorrectly folded proteins include undesirable disulfide isomers (i.e., with incorrect disulfide pairs) or molecules with intermolecular disulfide pairs forming oligomers of the desired protein products.

Brief Summary Text - BSTX (11):

Specifically exemplified herein is a thioredoxin shufflease producible when an E. coli thioredoxin gene is **mutated** at codon 35 to change **cysteine** (TGC) to a codon coding for any of the other 19 amino acids, i.e., alanine, arginine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, **methionine,** phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. This alteration changes the dithiol thioredoxin compound to a monothiol compound which is referred to as thioredoxin shufflease.

US-PAT-NO: 4798885

DOCUMENT-IDENTIFIER: US 4798885 A

TITLE: Compositions of hormonally active human and porcine inhibin containing an .alpha. chain and 62 chain

DATE-ISSUED: January 17, 1989

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mason; Anthony J.	San Francisco	CA	N/A	N/A
Seeburg; Peter H.	San Francisco	CA	N/A	N/A

APPL-NO: 06/ 906729

DATE FILED: December 31, 1986

PARENT-CASE:

This is a continuation-in-part of U.S. Ser. No. 827,710, filed Feb. 7, 1986, now abandoned, which in turn is a continuation-in-part of U.S. Ser. No. 783,910, filed Oct. 3, 1985, now abandoned.

US-CL-CURRENT: 530/350, 930/10 , 930/260 , 930/DIG.530 , 930/DIG.821

ABSTRACT:

DNA encoding the prepro inhibin .alpha. and .beta. chains has been isolated. This DNA is ligated into expression vectors and used to transform host cells for the preparation of inhibin or activin. Also provided are prohormone domains and other inhibin .alpha. or .beta. chain derivatives having therapeutic or diagnostic interest. The compositions provided herein are useful in the manipulation of fertility in animals.

24 Claims, 24 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 24

---------- KWIC ---------

Detailed Description Text - DETX (16):

Insertional and deletional amino acid sequence variants are proteins in which one or more amino acid residues are introduced into or removed from a predetermined site in the target inhibin, activin, prodomain or proform of inhibin or activin. Most commonly, insertional variants are fusions of

heterologous proteins or polypeptides to the amino or carboxyl terminus of the .alpha. or .beta. chains, the prodomains or other inhibin derivatives. Immunogenic derivatives are made by fusing an immunogenic polypeptide to the target sequence, e.g. a prodomain polypeptide, by synthesis in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Such immunogenic polypeptides preferably are bacterial polypeptides such as trpLE, betagalactosidase and the like, together with their immunogenic fragments. Other insertions entail inserting heterologous eukaryotic (e.g. the herpes virus gD signal) or microbial secretion signal or protease processing sequences upstream from the NH.sub.2 -terminus of the protein to be secreted. Deletions of cysteine or other labile residues also may be desirable, for example in increasing the **oxidative stability** of the .alpha. or .beta. chain. Deletional derivatives will produce .alpha. or .beta. chain fragments. Such fragments, when biologically or immunologically active, are within the scope herein. For instance, a fragment comprising .beta..sub.B or .beta..sub.A residues about from 11 to 45 (numbered from mature Gly.sub.1) is to be included within the scope herein.

Detailed Description Text - DETX (18):

Substitution derivatives are produced by **mutating** the DNA in a target codon, so that thereafter a different amino acid is encoded by the codon, with no concomitant change in the number of residues present in the molecule expressed from the **mutated** DNA. Substitutions or deletions are useful for example in increasing the stability of the proteins herein by eliminating proteolysis sites, wherein residues are substituted within or adjacent to the sites or are deleted from the sites, or by introducing additional disulfide bonds through the substitution of **cysteine** for other residues. Substitutions are useful for facilitating the synthesis or recovery of mature or prodomain .alpha. or .beta. chains. For example, **methionine** residues within the mature inhibin sequences are substituted or deleted, prepro sequences delted, **methionine** is inserted at the -1 site immediately NH.sub.2 terminal to the mature NH.sub.2 terminal residue and another sequence inserted N-terminal to the exogenous **methionine**. The inhibin derivative in this case is expressed as a fusion having an intermediate methionyl residue, which in turn is cleaved at this residue by cyanogen bromide in accordance with known practice. The mature inhibin derivative released from the fusion is recovered.

US-PAT-NO: 4760025

DOCUMENT-IDENTIFIER: US 4760025 A

TITLE: Modified enzymes and methods for making same

DATE-ISSUED: July 26, 1988

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Estell; David A.	Mountain View	CA	N/A	N/A
Wells; James A.	San Mateo	CA	N/A	N/A

APPL-NO: 06/ 614612

DATE FILED: May 29, 1984

US-CL-CURRENT: 510/392, 435/221 , 435/222 , 510/320 , 510/530 , 930/200 , 930/240

ABSTRACT:

A cloned subtilisin gene has been modified at specific sites to cause amino acid substitutions at certain spots in the enzyme. The modified enzyme, preferably produced by Bacillus, is useful in combination with detergents.

3 Claims, 18 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 16

---------- KWIC ---------

Brief Summary Text - BSTX (12):

Enzymes having characteristics which vary from available stock are required. In particular, enzymes having enhanced **oxidation stability** will be useful in extending the shelf life and bleach compatibility of proteases used in laundry products. Similarly, reduced **oxidation stability** would be useful in industrial processes that require the rapid and efficient quenching of enzymatic activity.

Brief Summary Text - BSTX (29):

Mutant enzymes are recovered which exhibit **oxidative stability** and/or pH-activity profiles which differ from the precursor enzymes. Procaryotic carbonyl hydrolases having varied Km, Kcat, Kcat/Km ratio and substrate specificity also are provided herein.

Drawing Description Text - DRTX (16):
FIG. 14 shows the enhanced **oxidation stability** of a subtilisin mutant.

Detailed Description Text - DETX (24):
The mutant carbonyl hydrolases expressed upon transformation of the suitable hosts are screened for enzymes exhibiting desired characteristics, e.g. substrate specificity, **oxidation stability,** pH-activity profiles and the like.

Detailed Description Text - DETX (27):
Oxidation stability is a further objective which is accomplished by **mutants** described in the examples. The stability may be enhanced or diminished as is desired for various uses. Enhanced stability is effected by deleting one or more **methionine,** tryptophan, **cysteine** or lysine residues and, optionally, substituting another amino acid residue not one of **methionine,** tryptophan, **cysteine** or lysine. The opposite substitutions result in diminished **oxidation stability**. The substituted residue is preferably alanyl, but neutral residues also are suitable.

Detailed Description Text - DETX (124):
B. subtilis strain BG2036 obtained by the method of Example 11 was transformed by the plasmids of Examples 14, 15 or 20 and by pS4-5 as a control. Transformants were plated or cultured in shaker flasks for 16 to 48 h at 37.degree. C. in LB media plus 12.5 .mu.g/ml chloramphenicol. Mutant enzymatically active subtilisin was recovered by dialyzing cell broth against 0.01M sodium phosphate buffer, pH 6.2. The dialyzed broth was then titrated to pH 6.2 with 1N HCl and loaded on a 2.5.times.2 cm column of CM cellulose (CM-52 Whatman). After washing with 0.01M sodium phosphate, pH 6.2, the subtilisins (except mutants at position +222) were eluted with the same buffer made 0.08N in NaCl. The mutant subtilisins at position +222 were each eluted with 0.1M sodium phosphate, pH 7.0. The purified mutant and wild type enzymes were then used in studies of **oxidation stability,** Km, Kcat, Kcat/Km ratio, pH optimum, and changes in substrate specificity.

Detailed Description Text - DETX (126):
Mutant Subtilisin Exhibiting Improved **Oxidation Stability**

Detailed Description Text - DETX (128):
To a total volume of 400 .mu.l of 0.1M, pH 7, NaPO.sub.4 buffer containing the indicated bleach concentrations (FIG. 14) sufficient enzyme was added to give a final concentration of 0.016 mg/ml of enzyme. The solutions were incubated at 25.degree. C. for 10 min. and assayed for enzyme activity as follows: 120 .mu.l of either ala+222 or wild type, or 100 .mu.l of the cys+222 incubation mixture was combined with 890 .mu.l 0.1M tris buffer at pH 8.6 and 10 .mu.l of a sAAPFpN (Example 18) substrate solution (20 mg/ml in DMSO). The rate of increase in absorbance at 410 nm due to release of p-nitroaniline (Del Mar, E.G., et al., 1979 "Anal. Biochem." 99, 316-320) was monitored. The

results are shown in FIG. 14. The alanine substitution produced considerably more stable enzyme than either the wild-type enzyme or a **mutant** in which a labile **cysteine** residue was substituted for **methionine**. Surprisingly, the alanine substitution did not substantially interfere with enzyme activity B against the assay substrate, yet conferred relative **oxidati_n stability** on the enzyme. The serine+222 **mutant** also exhibited improved **oxidation stability**.

US-PAT-NO: 6329444

DOCUMENT-IDENTIFIER: US 6329444 B1

TITLE: Dip-molded medical devices from cis-1,4-polyisoprene

DATE-ISSUED: December 11, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
McGlothlin; Mark W.	San Diego	CA	N/A	N/A
Schmid; Eric V.	San Diego	CA	N/A	N/A

APPL-NO: 09/ 172965

DATE FILED: October 14, 1998

US-CL-CURRENT: 523/105, 2/161.7 , 522/159 , 522/60 , 523/300 , 524/571 , 524/579 , 525/332.5 , 528/932 , 604/96.01

ABSTRACT:

Medical devices of synthetic rubber are prepared from cis-1,4-polyisoprene by dip molding without the use of sulfur containing components. The devices have surprisingly favorable tensile characteristics despite what is known in regard to synthetic cis-1,4-polyisoprene. In addition, the absence of both the proteins present in natural rubber and the sulfur components that are typically used in vulcanization of both natural rubber and cis-1,4-polyisoprene of the prior art renders the devices freely usable without causing the user to suffer Type I or Type IV allergic reactions that typically arise from contact with natural rubber.

34 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX (12):

To summarize the collective teachings of the prior art, cis-1,4-polyisoprene without the protein that is retained from natural rubber sources is believed to be unsuitable for dip-molded medical devices since products made from deproteinized natural rubber lack the tensile characteristics that are important features of these devices, even those products made from deproteinized rubber that has been crosslinked by irradiation. This expectation is reinforced by molecular weight considerations, the isoprene in natural rubber has a high molecular weight component of from about 1,000,000 amu to about 2,500,000 amu (number average), while synthetic polyisoprene has a

considerably lower molecular weight with a number average ranging from about 250,000 amu to about 350,000 amu. A lower molecular weight polymer is expected to have lesser tensile properties, including tensile set values. Synthetic polyisoprene also has a lower degree of branching, lower symmetry, and lower intermolecular forces. All of these characteristics contribute to and affect the tensile properties of the polymer. Furthermore, the prior art distinctly avoids mention of any crosslinking method other than the use of sulfur-containing compounds. Thus, there is no disclosure in the prior art of a dip-molded product of synthetic cis-1,.sup.4 -polyisoprene that is both **protein-free and sulfur-free** and that has tensile characteristics that are acceptable for medical devices, particularly those that undergo an elastic expansion during use on the order of 100% (i.e., twice its unexpanded size) or greater.

Brief Summary Text - BSTX (14):

It has now been discovered that medical devices formed by dip-molding synthetic cis-1,4-polyisoprene that is both **protein-free and sulfur-free** have properties that make them suitable as substitutes for devices made of natural rubber, without any of the allergic reactions or other health problems associated with natural rubber. This is contrary to the teachings of the prior art which suggest that synthetic cis-1,4-polyisoprene is inherently inferior to its naturally-occurring counterpart obtained from Hevena brasiliensis, and which suggest that sulfur-based vulcanization is needed to achieve the type of performance required in the typical medical uses of these devices. Since synthetic cis-1,4-polyisoprene lacks the proteins present in natural rubber, the Type I allergic ("immediate") reaction, arising either on its own or aggravated by the irritant dermatitis reaction, is avoided entirely. By eliminating the sulfur-containing components, the Type IV allergic reaction is avoided as well, or at least reduced. In addition to eliminating these adverse factors, the resulting product offers unexpectedly good tensile set values (resiliency).

Claims Text - CLTX (1):

1. A medical device for use in contact with living human tissue, comprised of synthetic cis-1,4-polyisoprene, said device being both **protein-free and sulfur-free,** said device formed by dip-molding, and said cis-1,4-polyisoprene crosslinked subsequent to said dip-molding to an extent sufficient to achieve a tensile set value of less than 5%.

Claims Text - CLTX (21):

21. A surgical or medical examination glove comprised of synthetic cis-1,4-polyisoprene that is both **protein-free and sulfur-free,** and that has been formed by dip-molding from a latex of synthetic cis-1,4-polyisoprene and cured with a peroxide curing agent subsequent to dip-molding during immersion in a molten salt bath.

Claims Text - CLTX (22):

22. A surgical or medical examination glove comprised of synthetic cis-1,4-polyisoprene that is both **protein-free and sulfur-free,** and that has

been formed by dip-molding from a latex of synthetic cis-1,4-polyisoprene, said latex having been partially cured by radiation prior to said dip-molding in the substantial absence of chemical crosslinking radiation agents and further cured by radiation subsequent to dip-molding.

Claims Text - CLTX (25):

25. A surgical or medical examination glove comprised of synthetic cis-1,4-polyisoprene that is both **protein-free and sulfur-free,** and that has been formed by dip-molding from a latex of synthetic cis-1,4-polyisoprene, said latex having been partially cured by radiation prior to said dip-molding in the presence of a sensitizing amount of a chemical radiation sensitizing agent and further cured by radiation subsequent to dip-molding.

Claims Text - CLTX (29):

29. A surgical or medical examination glove comprised of synthetic cis-1,4-polyisoprene that is both **protein-free and sulfur-free,** and that has been formed by dip-molding from a latex of synthetic cis-1,4-polyisoprene, said latex having been partially cured by a peroxide curing agent prior to said dip-molding and further cured by said peroxide curing agent subsequent to dip-molding.

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 * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 10:14:45 ON 18 JUL 2003

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COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 10:15:00 ON 18 JUL 2003
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

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FILE 'MEDLINE'
         41027 METHIONINE
         53229 CYSTEINE
        411577 MUTA?
L1         520 METHIONINE AND CYSTEINE AND MUTA?

FILE 'SCISEARCH'
         23176 METHIONINE
         37983 CYSTEINE
        388804 MUTA?
L2         319 METHIONINE AND CYSTEINE AND MUTA?

FILE 'LIFESCI'
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        185562 MUTA?
L3         178 METHIONINE AND CYSTEINE AND MUTA?

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L4           89 METHIONINE AND CYSTEINE AND MUTA?

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TOTAL FOR ALL FILES
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=> s l12 and oxidat?(3a)stab?
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           693 OXIDAT?(3A)STAB?
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        613872 STAB?
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           191 OXIDAT?(3A)STAB?
L15          1 L3 AND OXIDAT?(3A)STAB?

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                 (OXIDAT? OR OXIDN)
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TOTAL FOR ALL FILES
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L25         64 SULFUR FREE
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FILE 'SCISEARCH'
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L26        159 SULFUR FREE
               (SULFUR(W)FREE)

FILE 'LIFESCI'
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L27         25 SULFUR FREE
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                  (SULFUR(W)FREE)

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L33          17 SULFUR FREE
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FILE 'BIOTECHNO'
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L35          87 SULFUR FREE
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TOTAL FOR ALL FILES
L36        1474 SULFUR FREE

=> s l36 and l12
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L37           0 L25 AND L1

FILE 'SCISEARCH'
L38           0 L26 AND L2

FILE 'LIFESCI'
L39           0 L27 AND L3

FILE 'BIOTECHDS'
L40           0 L28 AND L4

FILE 'BIOSIS'
L41           0 L29 AND L5

FILE 'EMBASE'
L42           0 L30 AND L6

FILE 'HCAPLUS'
L43           2 L31 AND L7

FILE 'NTIS'
L44           0 L32 AND L8
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FILE 'ESBIOBASE'
L45           0 L33 AND L9

FILE 'BIOTECHNO'
L46           0 L34 AND L10

FILE 'WPIDS'
L47           0 L35 AND L11

TOTAL FOR ALL FILES
L48           2 L36 AND L12

=> s muta? and oxidat?(3a)stab?
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        165700 OXIDAT?
        327066 STAB?
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           668 OXIDAT?(3A)STAB?
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        656809 OXIDN
        898735 OXIDAT?
                 (OXIDAT? OR OXIDN)
       1320750 STAB?
         16195 OXIDAT?(3A)STAB?
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L55         117 MUTA? AND OXIDAT?(3A)STAB?

FILE 'NTIS'
           9404 MUTA?
          25148 OXIDAT?
         105724 STAB?
            619 OXIDAT?(3A)STAB?
L56           0 MUTA? AND OXIDAT?(3A)STAB?

FILE 'ESBIOBASE'
         190505 MUTA?
          54734 OXIDAT?
         127746 STAB?
            453 OXIDAT?(3A)STAB?
L57          36 MUTA? AND OXIDAT?(3A)STAB?

FILE 'BIOTECHNO'
         227060 MUTA?
          39899 OXIDAT?
         101756 STAB?
            250 OXIDAT?(3A)STAB?
L58          33 MUTA? AND OXIDAT?(3A)STAB?

FILE 'WPIDS'
          21713 MUTA?
         121941 OXIDAT?
          62023 OXIDN
             89 OXIDNS
         150369 OXIDAT?
                  (OXIDAT? OR OXIDN OR OXIDNS)
         674249 STAB?
           6117 OXIDAT?(3A)STAB?
L59          43 MUTA? AND OXIDAT?(3A)STAB?

TOTAL FOR ALL FILES
L60         521 MUTA? AND OXIDAT?(3A) STAB?

=> s l36(5a)(protein# or enzyme#)
FILE 'MEDLINE'
        1512283 PROTEIN#
         655549 ENZYME#
L61           9 L25(5A)(PROTEIN# OR ENZYME#)

FILE 'SCISEARCH'
        1200050 PROTEIN#
         403682 ENZYME#
L62           5 L26(5A)(PROTEIN# OR ENZYME#)

FILE 'LIFESCI'
         462393 PROTEIN#
         182410 ENZYME#
L63           3 L27(5A)(PROTEIN# OR ENZYME#)

FILE 'BIOTECHDS'
         109150 PROTEIN#
         106297 ENZYME#
L64           0 L28(5A)(PROTEIN# OR ENZYME#)

FILE 'BIOSIS'
        1509153 PROTEIN#
         697524 ENZYME#
L65           7 L29(5A)(PROTEIN# OR ENZYME#)

FILE 'EMBASE'
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       1187365 PROTEIN#
        686953 ENZYME#
L66          5 L30(5A)(PROTEIN# OR ENZYME#)

FILE 'HCAPLUS'
       1767653 PROTEIN#
        852791 ENZYME#
L67         11 L31(5A)(PROTEIN# OR ENZYME#)

FILE 'NTIS'
         17007 PROTEIN#
         11763 ENZYME#
L68          0 L32(5A)(PROTEIN# OR ENZYME#)

FILE 'ESBIOBASE'
        530893 PROTEIN#
        188101 ENZYME#
L69          2 L33(5A)(PROTEIN# OR ENZYME#)

FILE 'BIOTECHNO'
        607140 PROTEIN#
        330428 ENZYME#
L70          2 L34(5A)(PROTEIN# OR ENZYME#)

FILE 'WPIDS'
        112911 PROTEIN#
         65581 ENZYME#
L71          1 L35(5A)(PROTEIN# OR ENZYME#)

TOTAL FOR ALL FILES
L72         45 L36(5A)(PROTEIN# OR ENZYME#)

=> s l36 and oxidat?(3a)stab?
FILE 'MEDLINE'
        165700 OXIDAT?
        327066 STAB?
           693 OXIDAT?(3A)STAB?
L73          0 L25 AND OXIDAT?(3A)STAB?

FILE 'SCISEARCH'
        292198 OXIDAT?
        613872 STAB?
          3690 OXIDAT?(3A)STAB?
L74          0 L26 AND OXIDAT?(3A)STAB?

FILE 'LIFESCI'
         33477 OXIDAT?
        100268 STAB?
           191 OXIDAT?(3A)STAB?
L75          0 L27 AND OXIDAT?(3A)STAB?

FILE 'BIOTECHDS'
          8100 OXIDAT?
         31207 STAB?
           113 OXIDAT?(3A)STAB?
L76          0 L28 AND OXIDAT?(3A)STAB?

FILE 'BIOSIS'
        179952 OXIDAT?
        364253 STAB?
          1844 OXIDAT?(3A)STAB?
L77          0 L29 AND OXIDAT?(3A)STAB?

FILE 'EMBASE'
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        140717 OXIDAT?
        310173 STAB?
           668 OXIDAT?(3A)STAB?
L78          0 L30 AND OXIDAT?(3A)STAB?

FILE 'HCAPLUS'
        528623 OXIDAT?
        656809 OXIDN
        898735 OXIDAT?
                 (OXIDAT? OR OXIDN)
       1320750 STAB?
         16195 OXIDAT?(3A)STAB?
L79          3 L31 AND OXIDAT?(3A)STAB?

FILE 'NTIS'
         25148 OXIDAT?
        105724 STAB?
           619 OXIDAT?(3A)STAB?
L80          1 L32 AND OXIDAT?(3A)STAB?

FILE 'ESBIOBASE'
         54734 OXIDAT?
        127746 STAB?
           453 OXIDAT?(3A)STAB?
L81          0 L33 AND OXIDAT?(3A)STAB?

FILE 'BIOTECHNO'
         39899 OXIDAT?
        101756 STAB?
           250 OXIDAT?(3A)STAB?
L82          0 L34 AND OXIDAT?(3A)STAB?

FILE 'WPIDS'
        121941 OXIDAT?
         62023 OXIDN
            89 OXIDNS
        150369 OXIDAT?
                 (OXIDAT? OR OXIDN OR OXIDNS)
        674249 STAB?
          6117 OXIDAT?(3A)STAB?
L83          4 L35 AND OXIDAT?(3A)STAB?

TOTAL FOR ALL FILES
L84          8 L36 AND OXIDAT?(3A) STAB?

=> s (124 or 148 or 160 or 172 or 184) not 2001-2003/py
FILE 'MEDLINE'
       1311832 2001-2003/PY
L85         40 (L13 OR L37 OR L49 OR L61 OR L73) NOT 2001-2003/PY

FILE 'SCISEARCH'
       2383340 2001-2003/PY
L86         48 (L14 OR L38 OR L50 OR L62 OR L74) NOT 2001-2003/PY

FILE 'LIFESCI'
        223576 2001-2003/PY
L87         23 (L15 OR L39 OR L51 OR L63 OR L75) NOT 2001-2003/PY

FILE 'BIOTECHDS'
         48189 2001-2003/PY
L88         37 (L16 OR L40 OR L52 OR L64 OR L76) NOT 2001-2003/PY

FILE 'BIOSIS'
       1268117 2001-2003/PY
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L89         46 (L17 OR L41 OR L53 OR L65 OR L77) NOT 2001-2003/PY

FILE 'EMBASE'
       1092333 2001-2003/PY
L90         34 (L18 OR L42 OR L54 OR L66 OR L78) NOT 2001-2003/PY

FILE 'HCAPLUS'
       2507812 2001-2003/PY
L91         77 (L19 OR L43 OR L55 OR L67 OR L79) NOT 2001-2003/PY

FILE 'NTIS'
         35365 2001-2003/PY
L92           1 (L20 OR L44 OR L56 OR L68 OR L80) NOT 2001-2003/PY

FILE 'ESBIOBASE'
        696498 2001-2003/PY
L93         21 (L21 OR L45 OR L57 OR L69 OR L81) NOT 2001-2003/PY

FILE 'BIOTECHNO'
        279495 2001-2003/PY
L94         25 (L22 OR L46 OR L58 OR L70 OR L82) NOT 2001-2003/PY

FILE 'WPIDS'
       2343180 2001-2003/PY
L95         18 (L23 OR L47 OR L59 OR L71 OR L83) NOT 2001-2003/PY

TOTAL FOR ALL FILES
L96        370 (L24 OR L48 OR L60 OR L72 OR L84) NOT 2001-2003/PY

=> dup rem l96
PROCESSING COMPLETED FOR L96
L97         170 DUP REM L96 (200 DUPLICATES REMOVED)

=> d tot

L97   ANSWER 1 OF 170  BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI    New triacylglycerol oils with high 1,3-dierucoyl-2-oleoylglycerol
      content, useful as vegetable oil additives, in hydraulic oil compositions
      and as lubricant additives;
         oil production via methyl N-nitroguanidine-mediated rape seed
         mutagenesis for use in food and other industry
AU    Kodali D R; Fan Z; DeBonte L R
AN    2000-06848  BIOTECHDS
PI    WO 2000007432 17 Feb 2000

L97  ANSWER 2 OF 170  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI   alpha-amylase mutants.
SO   Official Gazette of the United States Patent and Trademark Office Patents,
     (Nov. 7, 2000) Vol. 1240, No. 1, pp. No Pagination. e-file.
     ISSN: 0098-1133.
AU   Svendsen, Allan (1); Borchert, Torben Vedel; Bisg&&ANGrd-Frantzen, Henrik
AN   2001:257063  BIOSIS

L97  ANSWER 3 OF 170  HCAPLUS  COPYRIGHT 2003 ACS
TI   Biosynthetic prodn. method of L-aspartic acid using oxidation-
     stable maleate cis-trans isomerase mutant from Serratia
     marcescens
SO   Jpn. Kokai Tokkyo Koho, 13 pp.
     CODEN: JKXXAF
IN   Hatakeyama, Kazuhisa
AN   2000:342195  HCAPLUS
DN   133:3761
     PATENT NO.        KIND  DATE          APPLICATION NO.  DATE
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PI JP 2000139466 A2 20000523 JP 1998-317119 19981109

L97 ANSWER 4 OF 170 WPIDS (C) 2003 THOMSON DERWENT
TI Non-naturally occurring protein with insulin activity useful for treating type 1 and type 2 diabetes, comprising amino acid substitutions as compared to native human insulin and having enhanced stability.
PI WO 2000069901 A2 20001123 (200103)* EN 95p C07K014-00
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2000051444 A 20001205 (200113) C07K014-00
IN DAHIYAT, B I

L97 ANSWER 5 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Effects of cadmium on manganese peroxidase - Competitive inhibition of Mn-II **oxidation** and thermal **stabilization** of the enzyme
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (MAR 2000) Vol. 267, No. 6, pp. 1761-1769.
Publisher: BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD, OXFORD OX2 0NE, OXON, ENGLAND.
ISSN: 0014-2956.
AU Youngs H L; Sundaramoorthy M; Gold M H (Reprint)
AN 2000:267503 SCISEARCH

L97 ANSWER 6 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 1
TI A fap7 allele for elevated palmitate in soybean
SO CROP SCIENCE, (NOV-DEC 2000) Vol. 40, No. 6, pp. 1538-1542.
Publisher: CROP SCIENCE SOC AMER, 677 S SEGOE ROAD, MADISON, WI 53711 USA.
ISSN: 0011-183X.
AU Stoltzfus D L; Fehr W R (Reprint); Welke G A; Hammond E G; Cianzio S R
AN 2001:2201 SCISEARCH

L97 ANSWER 7 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Analysis of oxidation sensitivity of maleate cis-trans isomerase from Serratia marcescens
SO BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY, (JUL 2000) Vol. 64, No. 7, pp. 1477-1485.
Publisher: JAPAN SOC BIOSCI BIOTECHN AGROCHEM, JAPAN ACAD SOC CTR BLDG, 2-4-6 YAYOI BUNKYO-KU, TOKYO 113, JAPAN.
ISSN: 0916-8451.
AU Hatakeyama K; Goto M; Kobayashi M (Reprint); Terasawa M; Yukawa H
AN 2000:600401 SCISEARCH

L97 ANSWER 8 OF 170 MEDLINE DUPLICATE 2
TI Stabilization of NAD-dependent formate dehydrogenase from Candida boidinii by site-directed **mutagenesis** of cysteine residues.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (2000 Mar) 267 (5) 1280-9.
Journal code: 0107600. ISSN: 0014-2956.
AU Slusarczyk H; Felber S; Kula M R; Pohl M
AN 2000156230 MEDLINE

L97 ANSWER 9 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 3
TI Recent advances in metal carcinogenicity
SO PURE AND APPLIED CHEMISTRY, (JUN 2000) Vol. 72, No. 6, pp. 1007-1014.
Publisher: INT UNION PURE APPLIED CHEMISTRY, 104 TW ALEXANDER DR, PO BOX 13757, RES TRIANGLE PK, NC 27709-3757.
ISSN: 0033-4545.
AU Hartwig A (Reprint)
AN 2000:726488 SCISEARCH

L97 ANSWER 10 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Quantitative measurement of total and free 3-hydroxy fatty acids in serum or plasma samples: short-chain 3-hydroxy fatty acids are not esterified
SO JOURNAL OF INHERITED METABOLIC DISEASE, (NOV 2000) Vol. 23, No. 7, pp. 745-750.
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT,. NETHERLANDS.
ISSN: 0141-8955.
AU Jones P M (Reprint); Burlina A B; Bennett M J
AN 2000:847095 SCISEARCH

L97 ANSWER 11 OF 170 MEDLINE DUPLICATE 4
TI The crystal structure of a sulfurtransferase from Azotobacter vinelandii highlights the evolutionary relationship between the rhodanese and phosphatase enzyme families.
SO JOURNAL OF MOLECULAR BIOLOGY, (2000 May 12) 298 (4) 691-704.
Journal code: 2985088R. ISSN: 0022-2836.
AU Bordo D; Deriu D; Colnaghi R; Carpen A; Pagani S; Bolognesi M
AN 2000251047 MEDLINE

L97 ANSWER 12 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Effects of .pi.-stacking interactions and hydrogen bonding on the redox properties of short-chain acyl-CoA dehydrogenase.
SO Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), INOR-536 Publisher: American Chemical Society, Washington, D. C.
CODEN: 69CLAC
AU Pellett, Jackson D.; Becker, Donald F.; Saenger, Amy K.; Fuchs, James A.; Stankovich, Marian T.
AN 2000:331321 HCAPLUS

L97 ANSWER 13 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Protein engineering of bacterial alpha-amylases; alpha-amylase enzyme engineering; a review
SO Biochim.Biophys.Acta Protein Struct.Mol.Enzymol.; (2000) 1543, 2, 253-74
CODEN: 1901H
AU Nielsen J E; *Borchert T V
AN 2001-02404 BIOTECHDS

L97 ANSWER 14 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Lipase protein engineering; enzyme engineering; a review
SO Biochim.Biophys.Acta Protein Struct.Mol.Enzymol.; (2000) 1543, 2, 223-38
CODEN: 1901H
AU Svendsen A
AN 2001-02402 BIOTECHDS

L97 ANSWER 15 OF 170 MEDLINE DUPLICATE 5
TI Substitution of the critical methionine residues in trigonopsis variabilis D-amino acid oxidase with leucine enhances its resistance to hydrogen peroxide.
SO FEMS MICROBIOLOGY LETTERS, (2000 May 15) 186 (2) 215-9.
Journal code: 7705721. ISSN: 0378-1097.
AU Ju S S; Lin L L; Chien H R; Hsu W H
AN 2000263764 MEDLINE

L97 ANSWER 16 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Ligand-binding proteins: Their potential for application in systems for controlled delivery and uptake of ligands
SO PHARMACOLOGICAL REVIEWS, (JUN 2000) Vol. 52, No. 2, pp. 207-236.
Publisher: AMER SOC PHARMACOLOGY EXPERIMENTAL THERAPEUTICS, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998.
ISSN: 0031-6997.
AU DeWolf F A (Reprint); Brett G M

AN 2000:431362 SCISEARCH

L97 ANSWER 17 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 6
TI Characteristics and **mutagenicity** of fumes obtained from commercial edible oils
SO JOURNAL OF FOOD AND DRUG ANALYSIS, (JUN 2000) Vol. 8, No. 2, pp. 133-140. Publisher: NATL LABORATORIES FOODS DRUGS, DEPT HEALTH, EXECUTIVE YUAN, 161-2 KUEN YANG ST DR. ERICK T. SUEN, DEPUTY DIR., NANKANG, TAIPEI, TAIWAN.
ISSN: 1021-9498.
AU Wu S C (Reprint); Yen G C
AN 2000:518885 SCISEARCH

L97 ANSWER 18 OF 170 MEDLINE DUPLICATE 7
TI Thermal **stable** and **oxidation**-resistant variant of subtilisin E.
SO JOURNAL OF BIOTECHNOLOGY, (2000 Aug 25) 81 (2-3) 113-8.
Journal code: 8411927. ISSN: 0168-1656.
AU Yang Y; Jiang L; Zhu L; Wu Y; Yang S
AN 2000443550 MEDLINE

L97 ANSWER 19 OF 170 LIFESCI COPYRIGHT 2003 CSA
TI Comparison of the levels of 8-hydroxyguanine in DNA as measured by gas chromatography mass spectrometry following hydrolysis of DNA by Escherichia coli Fpg protein or formic acid
SO Nucleic Acids Research [Nucleic Acids Res.], (20000801) vol. 28, no. 15, pp. E75-e75.
ISSN: 0305-1048.
AU Rodriguez, H.; Jurado, J.; Laval, J.; Dizdaroglu, M.
AN 2000:108921 LIFESCI

L97 ANSWER 20 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Safflower plants having high levels of unsaturated fatty acids compared to the saturated fatty acid content;
safflower plant which produces oil with high oleic acid content, useful for infant formula food or as a coconut substitute with high **oxidative stability**
AU Weisker A C
AN 1999-10537 BIOTECHDS
PI US 5912416 15 Jun 1999

L97 ANSWER 21 OF 170 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 9
TI **Sulfur-free**, PAO-base lubricants with excellent anti-wear properties and superior thermal/**oxidation stability**
SO U.S., 6 pp.
CODEN: USXXAM
IN Wong, Chung-Lai
AN 1999:606960 HCAPLUS
DN 131:216404

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 5955403	A	19990921	US 1998-47053	19980324
SG 70676	A1	20000222	SG 1999-1124	19990316
EP 952207	A2	19991027	EP 1999-302071	19990318
EP 952207	A3	20000419		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 11323367	A2	19991126	JP 1999-78601	19990323

L97 ANSWER 22 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI A DNA encoding an **oxidation-stable** maleate-isomerase;
Serratia marcescens recombinant oxidation-resistant maleate-isomerase, useful for producing fumaric acid from malic acid

AN 1999-14876 BIOTECHDS
PI JP 11221083 17 Aug 1999

L97 ANSWER 23 OF 170 WPIDS (C) 2003 THOMSON DERWENT
TI Virginia-type Runner peanut plant with seed containing modified oleic acid and palmitic acid content useful for industrial and food applications.
PI US 5948954 A 19990907 (199946)* 10p A01H005-00
IN EIKENBERRY, E J; HORN, M E; ROMERO LANUZA, J E; SUTTON, J D

L97 ANSWER 24 OF 170 WPIDS (C) 2003 THOMSON DERWENT
TI Electrical insulation oil - comprises mineral oil base having specific **mutagenicity** index, sulphur content, dimethyl sulphoxide extract amount and aromaticity percentage.
PI JP 11185530 A 19990709 (199938)* 7p H01B003-20

L97 ANSWER 25 OF 170 MEDLINE
TI Coupled oxidation of heme covalently attached to cytochrome b562 yields a novel biliprotein.
SO BIOCHEMISTRY, (1999 Dec 21) 38 (51) 16847-56.
Journal code: 0370623. ISSN: 0006-2960.
AU Rice J K; Fearnley I M; Barker P D
AN 2000074535 MEDLINE

L97 ANSWER 26 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Comparison of local and global stability of an analogue of a disulfide-folding intermediate with those of the wild-type protein in bovine pancreatic ribonuclease A: Identification of specific regions of **stable** structure along the **oxidative** folding pathway
SO BIOCHEMISTRY, (14 DEC 1999) Vol. 38, No. 50, pp. 16432-16442.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.
ISSN: 0006-2960.
AU Laity J H; Montelione G T; Scheraga H A (Reprint)
AN 2000:4165 SCISEARCH

L97 ANSWER 27 OF 170 MEDLINE DUPLICATE 10
TI Selection for improved subtiligases by phage display.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Aug 17) 96 (17) 9497-502.
Journal code: 7505876. ISSN: 0027-8424.
AU Atwell S; Wells J A
AN 1999380546 MEDLINE

L97 ANSWER 28 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Influence of the oxygen at the C8 position on the intramolecular proton transfer in C8-oxidative guanine
SO Journal of Physical Chemistry A (1999), 103(5), 577-584
CODEN: JPCAFH; ISSN: 1089-5639
AU Gu, Jiande; Leszczynski, Jerzy
AN 1999:40764 HCAPLUS
DN 130:182157

L97 ANSWER 29 OF 170 MEDLINE DUPLICATE 11
TI Directed evolution of a fungal peroxidase.
SO NATURE BIOTECHNOLOGY, (1999 Apr) 17 (4) 379-84.
Journal code: 9604648. ISSN: 1087-0156.
AU Cherry J R; Lamsa M H; Schneider P; Vind J; Svendsen A; Jones A; Pedersen A H
AN 1999224295 MEDLINE

L97 ANSWER 30 OF 170 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Chemical modification and site-directed **mutagenesis** of methionine residues in recombinant human granulocyte colony-stimulating factor: Effect on stability and biological activity.
SO Archives of Biochemistry and Biophysics, (Feb. 1, 1999) Vol. 362, No. 1,

pp. 1-11.
ISSN: 0003-9861.
AU Lu, Hsieng S. (1); Fausset, Patricia R.; Narhi, Linda O.; Horan, Thomas; Shinagawa, Kyoko; Shimamoto, Grant; Boone, Thomas C.
AN 1999:131334 BIOSIS

L97 ANSWER 31 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Starch liquefaction;
using Bacillus licheniformis alpha-amylase **mutant** with improved **oxidative stability**
AU Barnett C C; Solheim L P; Mitchinson C; Power S D; Requadt C A
AN 1999-03126 BIOTECHDS
PI US 5849549 15 Dec 1998

L97 ANSWER 32 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New soyabeans producing oil of low linolenic acid content with increased oxidation resistance;
soybean **mutagenesis**
AU Fehr W R; Hammond E G
AN 1998-08848 BIOTECHDS
PI WO 9827807 2 Jul 1998

L97 ANSWER 33 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Use of specific alpha-amylase enzymes;
enzyme engineering for application in laundry surfactant composition
AU Baeck A C; Jones L A; Ohtani R; Pramod K; Rai S; Showell M S
AN 1998-05749 BIOTECHDS
PI WO 9805748 12 Feb 1998

L97 ANSWER 34 OF 170 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 13
TI Enzymatic processing of uracil glycol, a major oxidative product of DNA cytosine.
SO Journal of Biological Chemistry, (17 Apr 1998) 273/16 (10026-10035).
Refs: 53
ISSN: 0021-9258 CODEN: JBCHA3
AU Purmal A.A.; Lampman G.W.; Bond J.P.; Hatahet Z.; Wallace S.S.
AN 1998134038 EMBASE

L97 ANSWER 35 OF 170 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 14
TI Genomic instability and catalase gene amplification induced by chronic exposure to oxidative stress.
SO Cancer Research, (1 Sep 1998) 58/17 (3986-3992).
Refs: 47
ISSN: 0008-5472 CODEN: CNREA8
AU Hunt C.R.; Sim J.E.; Sullivan S.J.; Featherstone T.; Golden W.; Von Kapp-Herr C.; Hock R.A.; Gomez R.A.; Parsian A.J.; Spitz D.R.
AN 1998297988 EMBASE

L97 ANSWER 36 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI A transposable partitioning locus used to **stabilize** plasmid-borne hydrogen **oxidation** and trifolitoxin production genes in a Sinorhizobium strain
SO Applied and Environmental Microbiology (1998), 64(5), 1657-1662
CODEN: AEMIDF; ISSN: 0099-2240
AU Kent, Angela D.; Wojtasiak, Michelle L.; Robleto, Eduardo A.; Triplett, Eric W.
AN 1998:271597 HCAPLUS
DN 129:63691

L97 ANSWER 37 OF 170 MEDLINE DUPLICATE 15
TI Chromium-induced genotoxicity and apoptosis: relationship to chromium carcinogenesis (review).
SO ONCOLOGY REPORTS, (1998 Nov-Dec) 5 (6) 1307-18. Ref: 84
Journal code: 9422756. ISSN: 1021-335X.

AU Singh J; Carlisle D L; Pritchard D E; Patierno S R
AN 1998443370 MEDLINE

L97 ANSWER 38 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI A comparison of the local and global effects of **mutations** on the stability of mammalian cytochrome c
SO Chemtracts (1998), 11(10), 713-728
CODEN: CHEMFW; ISSN: 1431-9268
AU Schejter, A.; Sanishvili, R.; Qin, W.; Margoliash, E.
AN 1998:622706 HCAPLUS
DN 129:340857

L97 ANSWER 39 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Cloning and stabilization of NAD-dependent formate dehydrogenase from Candida boidinii by site-directed **mutagenesis**
SO Progress in Biotechnology (1998), 15(Stability and Stabilization of Biocatalysis), 331-336
CODEN: PBITE3; ISSN: 0921-0423
AU Slusarczyk, H.; Pohl, M.; Kula, M.-R.
AN 1999:159947 HCAPLUS
DN 130:334599

L97 ANSWER 40 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Stability of the Escherichia coli ATP synthase F0F1 complex is dependent on interactions between .gamma.Gln-269 and the .beta. subunit loop .beta.Asp-301-.beta.Asp-305
SO Archives of Biochemistry and Biophysics (1998), 358(2), 277-282
CODEN: ABBIA4; ISSN: 0003-9861
AU Omote, Hiroshi; Tainaka, Ken-ichi; Fujie, Kazunari; Iwamoto-Kihara, Atsuko; Wada, Yoh; Futai, Masamitsu
AN 1998:678681 HCAPLUS
DN 130:22190

L97 ANSWER 41 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Stabilization of marine oils with flavonoids
SO JOURNAL OF FOOD LIPIDS, (SEP 1998) Vol. 5, No. 3, pp. 183-196.
Publisher: FOOD NUTRITION PRESS INC, 6527 MAIN ST, P O BOX 374, TRUMBULL, CT 06611.
ISSN: 1065-7258.
AU Wanasundara U N (Reprint); Shahidi F
AN 1998:792283 SCISEARCH

L97 ANSWER 42 OF 170 MEDLINE DUPLICATE 16
TI Effect of **mutations** in Candida antarctica B lipase.
SO CHEMISTRY AND PHYSICS OF LIPIDS, (1998 Jun) 93 (1-2) 95-101.
Journal code: 0067206. ISSN: 0009-3084.
AU Patkar S; Vind J; Kelstrup E; Christensen M W; Svendsen A; Borch K; Kirk O
AN 1998386721 MEDLINE

L97 ANSWER 43 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Humanized antibody binds the same cancer antigen as antibody NR-LU-13; humanized antibody for cancer diagnosis and immunotherapy; vector expression in maize cell for reduced immunogenicity or toxicity
AU Graves S S; Reno J M; Mallet R W; Hylarides M D; Searle S M J; Henry A H; Pedersen J T; Rees A R
AN 1998-02695 BIOTECHDS
PI WO 9746589 11 Dec 1997

L97 ANSWER 44 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Termamyl-like alpha-amylase variants with improved properties; enzyme engineering and expression in Bacillus spp.
AU Svensden A; Borchert T V; Bisgard-Frantzen H
AN 1998-01800 BIOTECHDS
PI WO 9741213 6 Nov 1997

L97 ANSWER 45 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Combined desizing and stone-washing of dyed denim;
using alpha-amylase, cellulase, endo-glucanase and optionally a thermostable lipase
AU Lund H
AN 1997-08349 BIOTECHDS
PI WO 9718286 22 May 1997

L97 ANSWER 46 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Production of improved rapeseed exhibiting an enhanced oleic acid content
SO U.S., 11 pp., Cont.-in-part of U.S. Ser. No. 140,137, abandoned.
CODEN: USXXAM
IN Wong, Raymond S. C.; Beversdorf, Wallace D.; Castagno, James R.; Grant, Ian; Patel, Jayantilal D.
AN 1997:410977 HCAPLUS
DN 127:94507

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 5638637	A	19970617	US 1988-286708	19881220
EP 323753	A1	19890712	EP 1988-312397	19881229
EP 323753	B1	19940824		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
ES 2063054	T3	19950101	ES 1988-312397	19881229
CA 1334289	A1	19950207	CA 1988-587196	19881229
US 5840946	A	19981124	US 1995-462904	19950605

L97 ANSWER 47 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Activation of recombinant proteins expressed in prokaryotic cells
SO Jpn. Kokai Tokkyo Koho, 13 pp.
CODEN: JKXXAF
IN Omae, Hiroaki; Suenaga, Masato; Nishimura, Tadashi
AN 1997:663985 HCAPLUS
DN 127:343598

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 09262093	A2	19971007	JP 1996-74775	19960328

L97 ANSWER 48 OF 170 WPIDS (C) 2003 THOMSON DERWENT
TI Ink composition useful for ink jet printing on paper, transparencies, fabric, etc. - comprises aqueous liquid vehicle, dye and lipid vesicles giving good water-fastness, light-fastness, thermal and **oxidative stability**, etc..

PI US 5626654	A	19970506 (199724)*	16p		C09D011-02
EP 778322	A2	19970611 (199728)	EN	14p	C09D011-00
R: DE FR GB					
JP 09176546	A	19970708 (199737)		11p	C09D011-02
EP 778322	A3	19971029 (199814)			C09D011-02
EP 778322	B1	20000517 (200028)	EN		C09D011-00
R: DE FR GB					
DE 69608397	E	20000621 (200037)			C09D011-00

IN BIRKEL, S; BRETON, M P; HAMER, G K; ISABELLA, M; NOOLANDI, J

L97 ANSWER 49 OF 170 MEDLINE
TI Nitric oxide trapping of the tyrosyl radical of prostaglandin H synthase-2 leads to tyrosine iminoxyl radical and nitrotyrosine formation.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Jul 4) 272 (27) 17086-90.
Journal code: 2985121R. ISSN: 0021-9258.
AU Gunther M R; Hsi L C; Curtis J F; Gierse J K; Marnett L J; Eling T E; Mason R P
AN 97347519 MEDLINE

L97 ANSWER 50 OF 170 MEDLINE DUPLICATE 17
TI Electrostatic effects on substrate activation in para-hydroxybenzoate

hydroxylase: studies of the **mutant** lysine 297 methionine.
SO BIOCHEMISTRY, (1997 Jun 17) 36 (24) 7548-56.
Journal code: 0370623. ISSN: 0006-2960.
AU Moran G R; Entsch B; Palfey B A; Ballou D P
AN 97344179 MEDLINE

L97 ANSWER 51 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Estrogens stabilize mitochondrial function and protect neural cells against the pro-apoptotic action of **mutant** presenilin-1
SO NeuroReport (1997), 8(17), 3817-3821
CODEN: NERPEZ; ISSN: 0959-4965
AU Mattson, Mark P.; Robinson, Nic; Guo, Qing
AN 1997:804642 HCAPLUS
DN 128:70935

L97 ANSWER 52 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Triacylglycerol composition and structure in genetically modified sunflower and soybean oils
SO JOURNAL OF THE AMERICAN OIL CHEMISTS SOCIETY, (AUG 1997) Vol. 74, No. 8, pp. 989-998.
Publisher: AMER OIL CHEMISTS SOC, 1608 BROADMOOR DRIVE, CHAMPAIGN, IL 61821-0489.
ISSN: 0003-021X.
AU Reske J; Siebrecht J; Hazebroek J (Reprint)
AN 97:622051 SCISEARCH

L97 ANSWER 53 OF 170 MEDLINE DUPLICATE 18.
TI Low linolenate and commercial soybean oils diminish serum HDL cholesterol in young free-living adult females.
SO JOURNAL OF THE AMERICAN COLLEGE OF NUTRITION, (1997 Dec) 16 (6) 562-9.
Journal code: 8215879. ISSN: 0731-5724.
AU Lu Z; Hendrich S; Shen N; White P J; Cook L R
AN 1998091919 MEDLINE

L97 ANSWER 54 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Alteration of the omega-3 fatty acid desaturase gene is associated with reduced linolenic acid in the A5 soybean genotype
SO THEORETICAL AND APPLIED GENETICS, (MAR 1997) Vol. 94, No. 3-4, pp. 356-359.
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
ISSN: 0040-5752.
AU Byrum J R; Kinney A J; Stecca K L; Grace D J; Diers B W (Reprint)
AN 97:284879 SCISEARCH

L97 ANSWER 55 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Studies on the structure and functions of HDL and apolipoprotein A-I of Beijing duck.
SO PROGRESS IN BIOCHEMISTRY AND BIOPHYSICS, (AUG 1997) Vol. 24, No. 4, pp. 334-339.
Publisher: SCIENCE CHINA PRESS, 16 DONGHUANGCHENGGEN NORTH ST, BEIJING 100717, PEOPLES R CHINA.
ISSN: 1000-3282.
AU Yin Y L (Reprint); Wang K Q; Chen B S
AN 97:674403 SCISEARCH

L97 ANSWER 56 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 19
TI Site 156 and 165 **mutation** of subtilisin E.
SO PROGRESS IN BIOCHEMISTRY AND BIOPHYSICS, (AUG 1997) Vol. 24, No. 4, pp. 331-334.
Publisher: SCIENCE CHINA PRESS, 16 DONGHUANGCHENGGEN NORTH ST, BEIJING 100717, PEOPLES R CHINA.
ISSN: 1000-3282.
AU Chen W D (Reprint); Ma J H; Zhu L Q
AN 97:674402 SCISEARCH

L97 ANSWER 57 OF 170 MEDLINE DUPLICATE 20
TI A small, **stable** RNA induced by **oxidative** stress: role as a pleiotropic regulator and antimutator.
SO CELL, (1997 Jul 11) 90 (1) 43-53.
Journal code: 0413066. ISSN: 0092-8674.
AU Altuvia S; Weinstein-Fischer D; Zhang A; Postow L; Storz G
AN 97373818 MEDLINE

L97 ANSWER 58 OF 170 LIFESCI COPYRIGHT 2003 CSA
TI Production of improved rapeseed exhibiting an enhanced oleic acid content
SO (19970617) . US Patent 5638637; US CLASS: 47/58; 47/DIG.1; 800/200; 800/230; 800/DIG.17; 800/DIG.69..
AU Wong, R.; Beversdorf, W.; Castagno, J.; Grant, I.; Patel, J.
AN 1999:88011 LIFESCI

L97 ANSWER 59 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New alpha-amylase variants; recombinant vector expression in bacterium or fungus for **mutant** enzyme production; application in surfactant composition etc.
AU Bisgard-Frantzen H; Svendsen A; Borchert T V
AN 1996-12566 BIOTECHDS
PI WO 9623873 8 Aug 1996

L97 ANSWER 60 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI An improved cleaning composition containing Bacillus licheniformis .alpha.-amylase **mutants** with improved thermal **stability** and **oxidation** resistance
SO PCT Int. Appl., 85 pp.
CODEN: PIXXD2
IN Barnett, Christopher C.; Mitchinson, Colin; Power, Scott D.
AN 1996:323628 HCAPLUS
DN 125:4407

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9605295	A2	19960222	WO 1995-US10426	19950809
	WO 9605295	A3	19960328		
	W: AU, BR, CA, CN, CZ, FI, HU, JP, KR, MX, NO, NZ, PL, RU, VN				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	CA 2197203	AA	19960222	CA 1995-2197203	19950809
	AU 9533662	A1	19960307	AU 1995-33662	19950809
	AU 686007	B2	19980129		
	EP 775201	A2	19970528	EP 1995-930186	19950809
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	CN 1158637	A	19970903	CN 1995-194852	19950809
	JP 10504197	T2	19980428	JP 1995-507603	19950809
	BR 9508582	A	19980602	BR 1995-8582	19950809
	HU 77748	A2	19980728	HU 1998-643	19950809
	FI 9700563	A	19970210	FI 1997-563	19970210
	NO 9700609	A	19970324	NO 1997-609	19970210

L97 ANSWER 61 OF 170 WPIDS (C) 2003 THOMSON DERWENT
TI Laundry detergent contg. a **mutant** alpha-amylase - has altered **oxidative stability**, a broadened pH performance profile and enhanced stability at either high or low temps..

PI	WO 9630481	A1	19961003 (199649)*	EN 105p	C11D003-386
	RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE				
	W: AU BR CA CN CZ FI HU JP KR MX NO NZ PL RO RU VN				
	AU 9653226	A	19961016 (199706)		C11D003-386
	EP 815193	A1	19980107 (199806)	EN	C11D003-386
	R: AT BE CH DE DK ES FI FR GB GR IE IT LI NL PT SE				
	NO 9704402	A	19971119 (199806)		C11D003-386
	BR 9607751	A	19980623 (199832)		C11D003-386

	MX 9706823	A1	19971101	(199902)		C11D003-386
	NZ 305257	A	19990128	(199910)		C11D003-386
	JP 11502562	W	19990302	(199919)	142p	C11D003-386
	KR 98702783	A	19980805	(199932)		C11D003-386
	AU 718509	B	20000413	(200028)		C11D003-386
	CN 1179176	A	19980415	(200220)		C11D003-386

IN BARNETT, C C; BOYER, S G; MITCHINSON, C; POWER, S D

L97 ANSWER 62 OF 170 MEDLINE DUPLICATE 21
TI Active site structural features for chemically modified forms of rhodanese.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Aug 30) 271 (35) 21054-61.
Journal code: 2985121R. ISSN: 0021-9258.
AU Gliubich F; Gazerro M; Zanotti G; Delbono S; Bombieri G; Berni R
AN 96355461 MEDLINE

L97 ANSWER 63 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI Specific recognition of A/G and A/7,8-dihydro-8-oxoguanine (8-oxoG) mismatches by Escherichia coli MutY: Removal of the C-terminal domain preferentially affects A/8-oxoG recognition
SO BIOCHEMISTRY, (24 DEC 1996) Vol. 35, No. 51, pp. 16665-16671.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.
ISSN: 0006-2960.
AU Gogos A; Cillo J; Clarke N D; Lu A L (Reprint)
AN 97:43397 SCISEARCH

L97 ANSWER 64 OF 170 MEDLINE DUPLICATE 22
TI Oxidative inactivation of thioredoxin as a cellular growth factor and protection by a Cys73-->Ser **mutation**.
SO BIOCHEMICAL PHARMACOLOGY, (1996 Dec 13) 52 (11) 1741-7.
Journal code: 0101032. ISSN: 0006-2952.
AU Gasdaska J R; Kirkpatrick D L; Montfort W; Kuperus M; Hill S R; Berggren M; Powis G
AN 97139536 MEDLINE

L97 ANSWER 65 OF 170 MEDLINE DUPLICATE 23
TI Phosphorylation of threonine 638 critically controls the dephosphorylation and inactivation of protein kinase Calpha.
SO CURRENT BIOLOGY, (1996 Sep 1) 6 (9) 1114-23.
Journal code: 9107782. ISSN: 0960-9822.
AU Bornancin F; Parker P J
AN 96398962 MEDLINE

L97 ANSWER 66 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Purification and crystallization of a subtilisin E **mutant**
SO Shengwu Huaxue Zazhi (1996), 12(5), 608-612
CODEN: SHZAE4; ISSN: 1000-8543
AU Lin, Zheng; Zhu, Liu-Qin; Ma, Jian-Hua; Bi, Ru-Chang
AN 1996:684568 HCAPLUS
DN 125:321317

L97 ANSWER 67 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 24
TI MAPPING LOCI CONTROLLING THE CONCENTRATIONS OF ERUCIC AND LINOLENIC ACIDS IN SEED OIL OF BRASSICA-NAPUS L
SO THEORETICAL AND APPLIED GENETICS, (JUL 1996) Vol. 93, No. 1-2, pp. 282-286.
ISSN: 0040-5752.
AU THORMANN C E; ROMERO J; MANTET J; OSBORN T C (Reprint)
AN 96:584462 SCISEARCH

L97 ANSWER 68 OF 170 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI Peroxidative hemolysis value as a prognostic parameter for **mutagenic** dangerous evaluation.
SO Mutation Research, (1996) Vol. 360, No. 3, pp. 265.

Meeting Info.: 25th Annual Meeting of the European Environmental Mutagen Society Noordwijkerhout, Netherlands June 18-23, 1995
ISSN: 0027-5107.
AU Khrypach, L.; Ingel, F.; Drobinskaya, I.; Krivtsova, L.; Revazova, J.
AN 1996:447207 BIOSIS

L97 ANSWER 69 OF 170 MEDLINE DUPLICATE 25
TI Congenital 6-phosphogluconate dehydrogenase (6PGD) deficiency associated with chronic hemolytic anemia in a Spanish family.
SO AMERICAN JOURNAL OF HEMATOLOGY, (1996 Dec) 53 (4) 221-7.
Journal code: 7610369. ISSN: 0361-8609.
AU Vives Corrons J L; Colomer D; Pujades A; Rovira A; Aymerich M; Merino A; Aguilar i Bascompte J L
AN 97105910 MEDLINE

L97 ANSWER 70 OF 170 Elsevier BIOBASE COPYRIGHT 2003 Elsevier Science B.V.
AN 1996027113 ESBIOBASE
TI Participation of nitric oxide and iron in the oxidation of DNA in asbestos-treated human lung epithelial cells
AU Chao C.-C.; Park S.-H.; Aust A.E.
CS A.E. Aust, Dept. of Chemistry and Biochemistry, Utah State University, Logan, UT 84322-0300, United States.
SO Archives of Biochemistry and Biophysics, (1996), 326/1 (152-157)
CODEN: ABBIA4 ISSN: 0003-9861
DT Journal; Article
CY United States
LA English
SL English

L97 ANSWER 71 OF 170 MEDLINE DUPLICATE 26
TI Thermal **stable** subtilisin with **oxidation**-resistant property.
SO ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1996 Oct 12) 799 82-4.
Journal code: 7506858. ISSN: 0077-8923.
AU Zhu L; Zhao Y; Bi R
AN 97116993 MEDLINE

L97 ANSWER 72 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 27
TI GENETIC-VARIABILITY OF TOCOPHEROL COMPOSITION IN SUNFLOWER SEEDS AS A BASIS OF BREEDING FOR IMPROVED OIL QUALITY
SO PLANT BREEDING, (APR 1996) Vol. 115, No. 1, pp. 33-36.
ISSN: 0179-9541.
AU DEMURIN Y (Reprint); SKORIC D; KARLOVIC D
AN 96:357261 SCISEARCH

L97 ANSWER 73 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI **Mutant** B. licheniformis alpha-amylase enzymes;
Bacillus licheniformis **mutant** thermostable enzyme production; application in starch degradation, textile or paper desizing, brewing industry and as household surfactant
AU van der Laan J M; Aehle W
AN 1996-03039 BIOTECHDS
PI WO 9535382 28 Dec 1995

L97 ANSWER 74 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Use of an **oxidation stable** alpha-amylase;
Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus stearothermophilus, Aspergillus oryzae, Aspergillus niger enzyme engineering for use in textile desizing and bleaching
AU Toft A H; Marcher D; Pedersen H H; Nilsson T E
AN 1995-12862 BIOTECHDS
PI WO 9521247 10 Aug 1995

L97 ANSWER 75 OF 170 HCAPLUS COPYRIGHT 2003 ACS

TI **Mutation** of .beta.-galactosidase fragments to improve **stability** and resistance to **oxidation** and their application in enzyme complementation immunoassay.
SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2
IN Eisenbeis, Scott J.; Krevolin, Mark; Bryant, Christopher P.; Boguslawski, Sophie J.; Ledden, David J.; Clark, Scott
AN 1995:682915 HCAPLUS
DN 123:192375

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9511965	A1	19950504	WO 1994-US12533	19941028
W: CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5464747	A	19951107	US 1993-146633	19931029
CA 2175060	AA	19950504	CA 1994-2175060	19941028
EP 736090	A1	19961009	EP 1994-932141	19941028
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
JP 09504179	T2	19970428	JP 1994-512891	19941028
JP 2974158	B2	19991108		

L97 ANSWER 76 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Influence of the Oxidatively Damaged Adduct 8-Oxodeoxyguanosine on the Conformation, Energetics, and Thermodynamic Stability of a DNA Duplex
SO Biochemistry (1995), 34(49), 16148-60
CODEN: BICHAW; ISSN: 0006-2960
AU Plum, G. Eric; Grollman, Arthur P.; Johnson, Francis; Breslauer, Kenneth J.
AN 1995:950152 HCAPLUS
DN 124:24084

L97 ANSWER 77 OF 170 MEDLINE DUPLICATE 28
TI Recombinant calpain II: improved expression systems and production of a C105A active-site **mutant** for crystallography.
SO PROTEIN ENGINEERING, (1995 Aug) 8 (8) 843-8.
Journal code: 8801484. ISSN: 0269-2139.
AU Elce J S; Hegadorn C; Gauthier S; Vince J W; Davies P L
AN 96227679 MEDLINE

L97 ANSWER 78 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 29
TI CHEMICAL-INSTABILITY OF PROTEIN PHARMACEUTICALS - MECHANISMS OF **OXIDATION** AND STRATEGIES FOR **STABILIZATION**
SO BIOTECHNOLOGY AND BIOENGINEERING, (05 DEC 1995) Vol. 48, No. 5, pp. 490-500.
ISSN: 0006-3592.
AU LI S H; SCHONEICH C; BORCHARDT R T (Reprint)
AN 95:801551 SCISEARCH

L97 ANSWER 79 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Characterization of in vitro oxidized barstar
SO FEBS Letters (1995), 370(3), 273-7
CODEN: FEBLAL; ISSN: 0014-5793
AU Frisch, C.; Schreiber, G.; Fersht, A. R.
AN 1995:791249 HCAPLUS
DN 123:192106

L97 ANSWER 80 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Investigation of .beta.-oxidation intermediates in normal and MCAD-deficient human fibroblasts using tandem mass spectrometry
SO Biochemical and Molecular Medicine (1995), 54(1), 59-66
CODEN: BMMEF4; ISSN: 1077-3150
AU Nada, Mohamed A.; Chace, Donald H.; Sprecher, Howard; Roe, Charles R.
AN 1995:469715 HCAPLUS
DN 122:234619

L97 ANSWER 81 OF 170 MEDLINE DUPLICATE 30
TI Mitochondrial and cytosolic rhodanese from liver of DAB treated mice. II. Some properties and spectral studies.
SO CANCER BIOCHEMISTRY BIOPHYSICS, (1995 Jun) 15 (1) 55-63.
Journal code: 7506524. ISSN: 0305-7232.
AU Vazquez E; Polo C; Batlle A M
AN 96083972 MEDLINE

L97 ANSWER 82 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Modified Pseudomonas lipase with 21 methionine replaced; Pseudomonas pseudoalcaligenes recombinant lipase production, and use in a surfactant composition
AU Ven Der Laan J M; Lenting H B M; Mulleners L J S M; Cox M M J
AN 1995-01230 BIOTECHDS
PI WO 9425578 10 Nov 1994

L97 ANSWER 83 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Bacillus licheniformis, Bacillus stearothermophilus and Bacillus amyloliquefaciens alpha-amylase enzyme engineering by site-directed **mutagenesis**; DNA sequence; application in a surfactant or a starch liquefaction composition
AN 1994-13784 BIOTECHDS
PI WO 9418314 18 Aug 1994

L97 ANSWER 84 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Preparation of 4-O-(.alpha.-D-glucopyranosyl)-4-hydroxy-3(2H)-furanone as antitumor and antimutagenic agents
SO Jpn. Kokai Tokkyo Koho, 7 pp.
CODEN: JKXXAF
IN Kitao, Satoru; Shimaoka, Yoko; Sekine, Hiroshi
AN 1994:558097 HCAPLUS
DN 121:158097

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	JP 06135987	A2	19940517	JP 1992-312701	19921029

L97 ANSWER 85 OF 170 WPIDS (C) 2003 THOMSON DERWENT
TI Recombinant modified elafin with improved **oxidation stability** - has pharmaceutical use as an elastase inhibitor.

PI	WO 9404697	A1 19940303 (199410)*	JA	35p	C12P021-02
	RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE				
	W: JP US				
	JP 06506098	X 19940901 (199439)			C12P021-02
	EP 662516	A1 19950712 (199532)	EN	21p	C12P021-02
	R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE				
	EP 662516	A4 19970129 (199722)			C12P021-02
	JP 2653247	B2 19970917 (199742)		13p	C07K014-81
	US 5734014	A 19980331 (199820)		18p	C07K007-00

IN AMAGAYA, S; ISHIMA, Y; KAJI, A; OKAWA, N; YOSHIDA, M

L97 ANSWER 86 OF 170 MEDLINE DUPLICATE 31
TI 5-Formyltetrahydropteroylpolyglutamates are the major folate derivatives in Neurospora crassa conidiospores.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Nov 18) 269 (46) 28757-63.
Journal code: 2985121R. ISSN: 0021-9258.
AU Kruschwitz H L; McDonald D; Cossins E A; Schirch V
AN 95050682 MEDLINE

L97 ANSWER 87 OF 170 MEDLINE DUPLICATE 32
TI Active site coordination chemistry of the cytochrome c peroxidase Asp235Ala variant: spectroscopic and functional characterization.
SO BIOCHEMISTRY, (1994 Jun 28) 33 (25) 7819-29.

Journal code: 0370623. ISSN: 0006-2960.
AU Ferrer J C; Turano P; Banci L; Bertini I; Morris I K; Smith K M; Smith M; Mauk A G
AN 94281213 MEDLINE

L97 ANSWER 88 OF 170 MEDLINE DUPLICATE 33
TI **Mutations** of noncatalytic sulfhydryl groups influence the **stability**, folding, and **oxidative** susceptibility of rhodanese.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Feb 4) 269 (5) 3423-8.
Journal code: 2985121R. ISSN: 0021-9258.
AU Miller-Martini D M; Chirgwin J M; Horowitz P M
AN 94148838 MEDLINE

L97 ANSWER 89 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Catalytic properties of Phe42 .fwdarw. His **mutant** of horseradish peroxidase expressed in E. coli
SO Izvestiya Akademii Nauk, Seriya Khimicheskaya (1994), (11), 2034-8
CODEN: IASKEA
AU Loginov, D. B.; Gazaryan, I. G.; Doseeva, V. V.; Galkin, A. G.; Tishkov, V. I.; Mareeva, E. A.; Orlova, M. A.
AN 1995:497961 HCAPLUS
DN 123:4456

L97 ANSWER 90 OF 170 MEDLINE DUPLICATE 34
TI **Mutation** of tyrosine-67 to phenylalanine in cytochrome c significantly alters the local heme environment.
SO JOURNAL OF MOLECULAR BIOLOGY, (1994 Jan 28) 235 (4) 1326-41.
Journal code: 2985088R. ISSN: 0022-2836.
AU Berghuis A M; Guillemette J G; Smith M; Brayer G D
AN 94141926 MEDLINE

L97 ANSWER 91 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 35
TI EFFECTS OF FRYING OIL COMPOSITION ON POTATO-CHIP STABILITY
SO JOURNAL OF THE AMERICAN OIL CHEMISTS SOCIETY, (OCT 1994) Vol. 71, No. 10, pp. 1117-1121.
ISSN: 0003-021X.
AU WARNER K (Reprint); ORR P; PARROTT L; GLYNN M
AN 94:668205 SCISEARCH

L97 ANSWER 92 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Increased nitrosamine and nitrate biosynthesis mediated by nitric oxide synthase induced in hamsters infected with liver fluke (Opisthorchis viverrini)
SO Carcinogenesis (1994), 15(2), 271-5
CODEN: CRNGDP; ISSN: 0143-3334
AU Ohshima, H.; Bandaletova, T. Y.; Brouet, I.; Bartsch, H.; Kirby, G.; Ogunbiyi, F.; Vatanasapt, V.; Pipitgool, V.
AN 1994:188558 HCAPLUS
DN 120:188558

L97 ANSWER 93 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI PERFORMANCE EVALUATION OF HEXANE-EXTRACTED OILS FROM GENETICALLY-MODIFIED SOYBEANS
SO JOURNAL OF THE AMERICAN OIL CHEMISTS SOCIETY, (FEB 1994) Vol. 71, No. 2, pp. 157-161.
ISSN: 0003-021X.
AU MOUNTS T L (Reprint); WARNER K; LIST G R
AN 94:130990 SCISEARCH

L97 ANSWER 94 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 36
TI MAJOR OXIDATIVE PRODUCTS OF CYTOSINE, 5-HYDROXYCYTOSINE AND 5-HYDROXYURACIL, EXHIBIT SEQUENCE CONTEXT-DEPENDENT MISPAIRING IN-VITRO
SO NUCLEIC ACIDS RESEARCH, (11 JAN 1994) Vol. 22, No. 1, pp. 72-78.

ISSN: 0305-1048.
AU PURMAL A A; KOW Y W; WALLACE S S (Reprint)
AN 94:69245 SCISEARCH

L97 ANSWER 95 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Stabilization of polyoxyalkylene polyether polyols for polyurethane foam
SO PCT Int. Appl., 32 pp.
CODEN: PIXXD2
IN Wheeler, Edward L.; Barry, Lawrence B.; Richardson, Mark C.
AN 1993:651352 HCAPLUS
DN 119:251352

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9305108	A1	19930318	WO 1992-US7227	19920826
W: BR, CA, CS, JP, KR, RU				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE				
EP 603341	A1	19940629	EP 1992-924451	19920826
R: AT, CH, DE, FR, GB, IT, LI				
JP 06506018	T2	19940707	JP 1993-505272	19920826
US 5268394	A	19931207	US 1993-15329	19930209

L97 ANSWER 96 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI MOLYBDENUM COFACTOR - ITS BIOLOGICAL SIGNIFICANCE, STRUCTURAL, AND SYNTHETIC ASPECTS
SO HETEROCYCLES, (01 MAY 1993) Vol. 35, No. 2, pp. 1551-1570.
ISSN: 0385-5414.
AU GOSWAMI S (Reprint)
AN 93:438781 SCISEARCH

L97 ANSWER 97 OF 170 MEDLINE DUPLICATE 37
TI Examination of alpha-carbonyl derivatives of nitrosodimethylamine and ethylnitrosomethylamine as putative proximate carcinogens.
SO CARCINOGENESIS, (1993 Jun) 14 (6) 1189-93.
Journal code: 8008055. ISSN: 0143-3334.
AU Elespuru R K; Saavedra J E; Kovatch R M; Lijinsky W
AN 93284693 MEDLINE

L97 ANSWER 98 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 38
TI INSTABILITY AND DECAY OF THE PRIMARY STRUCTURE OF DNA
SO NATURE, (22 APR 1993) Vol. 362, No. 6422, pp. 709-715.
ISSN: 0028-0836.
AU LINDAHL T (Reprint)
AN 93:246319 SCISEARCH

L97 ANSWER 99 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Stability of industrial enzymes;
enzyme stabilization by chemical modification or enzyme engineering (conference paper)
SO Stud.Org.Chem.; (1993) 47, 111-31
CODEN: 9999T
AU Misset O
AN 1994-05917 BIOTECHDS

L97 ANSWER 100 OF 170 MEDLINE DUPLICATE 39
TI Protein engineering of subtilisins to improve stability in detergent formulations.
SO JOURNAL OF BIOTECHNOLOGY, (1993 Mar) 28 (1) 55-68.
Journal code: 8411927. ISSN: 0168-1656.
AU von der Osten C; Branner S; Hastrup S; Hedegaard L; Rasmussen M D; Bisgard-Frantzen H; Carlsen S; Mikkelsen J M
AN 93221820 MEDLINE

L97 ANSWER 101 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Protein engineering of subtilisins to improve stability in detergent

formulations;
Bacillus lentus subtilisin enzyme engineering to improve storage and **oxidation stability**
SO J.Biotechnol.; (1993) 28, 1, 55-68
CODEN: JBITD4
AU von der Osten C; Branner S; Hastrup S; Hedegaard L; Rasmussen M D; Bisgard-Frantzen H
AN 1993-05304 BIOTECHDS

L97 ANSWER 102 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Protein engineering and design: method and the industrial relevance; including enzyme engineering; a review
SO J.Biotechnol.; (1993) 28, 1, 1-23
CODEN: JBITD4
AU Recktenwald A; Schomburg D; Schmid R D
AN 1993-04874 BIOTECHDS

L97 ANSWER 103 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Treatment of thrombotic disease using thrombomodulin analog; produced by Escherichia coli, Spodoptera frugiperda insect cell culture, CHO cell culture, etc.
AN 1992-06845 BIOTECHDS
PI WO 9203149 5 Mar 1992

L97 ANSWER 104 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Serine protease variants having improved peptide-ligase activity; site-directed **mutagenesis** enzyme engineering of Bacillus amyloliquefaciens subtilisin for use in ligation
AN 1992-06508 BIOTECHDS
PI WO 9202615 20 Feb 1992

L97 ANSWER 105 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 40
TI **OXIDATIVE STABILITY** OF SOYBEAN OILS WITH ALTERED FATTY-ACID COMPOSITIONS
SO JOURNAL OF THE AMERICAN OIL CHEMISTS SOCIETY, (JUN 1992) Vol. 69, No. 6, pp. 528-532.
ISSN: 0003-021X.
AU LIU H R; WHITE P J (Reprint)
AN 92:373907 SCISEARCH

L97 ANSWER 106 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 41
TI CONTINUOUS CHEMOTROPIC GROWTH AND RESPIRATION OF CHROMATIACEAE SPECIES AT LOW OXYGEN CONCENTRATIONS
SO ARCHIVES OF MICROBIOLOGY, (JUN 1992) Vol. 158, No. 1, pp. 59-67.
ISSN: 0302-8933.
AU OVERMANN J (Reprint); PFENNIG N
AN 92:548562 SCISEARCH

L97 ANSWER 107 OF 170 MEDLINE
TI Genetic **stability** and **oxidative** stress: common mechanisms in aging and cancer.
SO EXS, (1992) 62 31-46. Ref: 26
Journal code: 9204529.
AU Cutler R G
AN 93082010 MEDLINE

L97 ANSWER 108 OF 170 WPIDS (C) 2003 THOMSON DERWENT
TI New **oxidatively stable mutant** detergent enzymes - with **cysteine** thiol gps. converted to alkyl di thio gps..
PI WO 9116423 A 19911031 (199146)*
RW: AT BE CH DE DK ES FR GB GR IT LU NL SE
W: FI JP KR
FI 9204698 A 19921016 (199304) C12N000-00

EP 528864 A1 19930303 (199309) EN 27p C12N009-50
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE
US 5208158 A 19930504 (199319) 10p C12N009-50
JP 05507402 W 19931028 (199348) 9p C12N009-56
IN BECH, L M; BRANNER, S; BREDDAM, K; GR, N H; GROEN, H; GRON, H

L97 ANSWER 109 OF 170 WPIDS (C) 2003 THOMSON DERWENT
TI Glucosyl transferase inhibiting substance useful as anticaries agent - comprising protein of 4.5 isoelectric point and effective when contained in food contg. e.g. GTase inactivating ingredient.
PI JP 03017100 A 19910125 (199110)* 5p
JP 2857418 B2 19990217 (199912) 6p C07K014-47

L97 ANSWER 110 OF 170 WPIDS (C) 2003 THOMSON DERWENT
TI **Mutant** protease gene(s) and protease(s) - derived from type I and III protease genes from lactococcal strains, used in fermentation foodstuffs and flavourings.
PI EP 411715 A 19910206 (199106)*
WO 9102064 A 19910221 (199110)
NL 8902010 A 19910301 (199113)
AU 9060515 A 19910311 (199123)
ZA 9006137 A 19920429 (199222) 31p C12N
EP 494149 A1 19920715 (199229) EN 29p C12N015-57
AU 638042 B 19930617 (199331) C12N015-57
WO 9102064 A3 19910725 (199508)
JP 08503840 W 19960430 (199645) 53p C12N015-09
IN DEVOS, W M; HAANDRIKMA, A J; KOK, J; SIEZEN, R J; VENEMA, G; VOS, P A J; DE, VOS W; HAANDRIKMAN, A J; DE, VOS W M

L97 ANSWER 111 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Protein engineered detergent proteases; comparison of Bacillus amyloliquefaciens and Bacillus licheniformis subtilisin as surfactant enzymes and improvement by site-directed **mutagenesis**; enzyme engineering (conference abstract)
SO INFORM; (1991) 2, 4, 316
AU Showell M S; Venegas M G; Barnett B L; Wertz W C
AN 1991-07159 BIOTECHDS

L97 ANSWER 112 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Genetic modification of canola oil: high oleic acid canola
SO Advances in Applied Biotechnology Series (1991), 12(Fat Cholesterol Reduced Foods), 141-52
CODEN: AASEE6; ISSN: 1053-4490
AU Wong, Raymond Sze Chung; Swanson, Eric
AN 1992:406531 HCAPLUS
DN 117:6531

L97 ANSWER 113 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI **Mutant** prokaryotic carbonyl-hydrolase enzymes; lipase or protease e.g. Bacillus amyloliquefaciens or Bacillus subtilis subtilisin gene cloning and expression; enzyme engineering by site-directed **mutagenesis**; DNA probe DNA sequence
AN 1990-06581 BIOTECHDS
PI EP 357157 7 Mar 1990

L97 ANSWER 114 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Subtilisin analogs with improved stability for use in detergents
SO Eur. Pat. Appl., 39 pp.
CODEN: EPXXDW
IN Zukowski, Mark M.; Narhi, Linda O.; Levitt, Michael
AN 1991:467525 HCAPLUS
DN 115:67525

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

PI EP 398539 A1 19901122 EP 1990-304715 19900501
EP 398539 B1 19960814
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE
WO 9014420 A1 19901129 WO 1990-US2234 19900423
W: AU, JP, KR
AU 9055580 A1 19901218 AU 1990-55580 19900423
AU 618675 B2 19920102
JP 03505976 T2 19911226 JP 1990-507107 19900423
AT 141324 E 19960815 AT 1990-304715 19900501
ES 2091224 T3 19961101 ES 1990-304715 19900501
CA 2016211 AA 19901117 CA 1990-2016211 19900507
CA 2016211 C 19980721
US 5397705 A 19950314 US 1992-857714 19920325

L97 ANSWER 115 OF 170 WPIDS (C) 2003 THOMSON DERWENT
TI Mostly new 2,4-di aryl-5-alkyl-imidazole derivs. - useful as redox indicators, effective in presence of thiol cpds. esp. for creatine kinase assay.
PI DE 3917677 A 19901206 (199050)*
EP 402667 A 19901219 (199051)
R: AT BE CH DE ES FR GB GR IT LU NL SE
CA 2017146 A 19901130 (199108)
JP 03019699 A 19910128 (199110)
DD 297998 A5 19920130 (199226) C12Q001-50
US 5162200 A 19921110 (199248) 9p C12Q001-50
US 5274095 A 19931228 (199401) 12p C07D401-04
EP 402667 B1 19940202 (199405) DE 27p C07D233-64
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE
DE 59004469 G 19940317 (199412) C07D233-64
ES 2062171 T3 19941216 (199505) C07D233-64
JP 07047586 B2 19950524 (199525) 13p C07D455-04
IN BRAUN, H P; DENEKE, U; GUETHLEIN, W; NAGEL, R; BRAUN, H

L97 ANSWER 116 OF 170 MEDLINE DUPLICATE 43
TI A highly active and oxidation-resistant subtilisin-like enzyme produced by a combination of site-directed **mutagenesis** and chemical modification.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1990 Dec 27) 194 (3) 897-901.
Journal code: 0107600. ISSN: 0014-2956.
AU Gron H; Bech L M; Branner S; Breddam K
AN 91099373 MEDLINE

L97 ANSWER 117 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI A HIGHLY-ACTIVE AND OXIDATION-RESISTANT SUBTILISIN-LIKE ENZYME PRODUCED BY A COMBINATION OF SITE-DIRECTED **MUTAGENESIS** AND CHEMICAL MODIFICATION
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1990) Vol. 194, No. 3, pp. 897-901.
AU GRON H; BECH L M; BRANNER S; BREDDAM K (Reprint)
AN 91:82486 SCISEARCH

L97 ANSWER 118 OF 170 LIFESCI COPYRIGHT 2003 CSA
TI A highly active and oxidation-resistant subtilisin-like enzyme produced by a combination of site-directed **mutagenesis** and chemical modification.
SO EUR. J. BIOCHEM., (1990) vol. 194, no. 3, pp. 897-901.
AU Groen, H.; Bech, L.M.; Branner, S.; Breddam, K.
AN 93:73466 LIFESCI

L97 ANSWER 119 OF 170 MEDLINE DUPLICATE 44
TI Mechanism of action of urocanase. Specific 13C-labelling of the prosthetic NAD+ and revision of the structure of its adduct with imidazolylpropionate.
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1990 Sep 24) 192 (3) 669-76.
Journal code: 0107600. ISSN: 0014-2956.

AU Klepp J; Fallert-Muller A; Grimm K; Hull W E; Retey J
AN 91006159 MEDLINE

L97 ANSWER 120 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISI
TI MECHANISM OF ACTION OF UROCANASE - SPECIFIC C-13-LABELING OF THE PROSTHETIC NAD+ AND REVISION OF THE STRUCTURE OF ITS ADDUCT WITH IMIDAZOLYLPROPIONATE
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1990) Vol. 192, No. 3, pp. 669-676.
AU KLEPP J; FALLERTMULLER A; GRIMM K; HULL W E; RETEY J (Reprint)
AN 91:58521 SCISEARCH

L97 ANSWER 121 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI **Oxidative** and flavor **stability** of edible refined oil prepared from lipoxygenase isoenzymes null soybeans
SO Yukagaku (1990), 39(9), 618-21
CODEN: YKGKAM; ISSN: 0513-398X
AU Endo, Yasushi; Endo, Hiromi; Fujimoto, Kenshiro; Kitamura, Keisuke
AN 1990:590033 HCAPLUS
DN 113:190033

L97 ANSWER 122 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI **Mutant** recombinant subtilisin enzymes; with improved **stability** to **oxidation**, increased proteolytic activity or improved washability; DNA sequence
AN 1989-12861 BIOTECHDS
PI WO 8906279 13 Jul 1989

L97 ANSWER 123 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Adaptive eradication of **methionine** and **cysteine** from cyanobacterial light-harvesting proteins
SO Nature (London, United Kingdom) (1989), 341(6239), 245-8
CODEN: NATUAS; ISSN: 0028-0836
AU Mazel, Didier; Marliere, Philippe
AN 1990:230444 HCAPLUS
DN 112:230444

L97 ANSWER 124 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Purification of coal-chemical industry effluents; using microorganisms able to degrade phenolic waste and grown in continuous culture and then in batch culture
AN 1988-10549 BIOTECHDS
PI SU 1364608 7 Jan 1988

L97 ANSWER 125 OF 170 LIFESCI COPYRIGHT 2003 CSA DUPLICATE 46
TI Preparation, toxicity and **mutagenicity** of 1-methyl-2-nitrosoimidazole. A toxic 2-nitroimidazole reduction product.
SO BIOCHEM. PHARMACOL., (1988) vol. 37, no. 13, pp. 2585-2593.
AU Noss, M.B.; Panicucci, R.; McClelland, R.A.; Rauth, A.M.
AN 88:106381 LIFESCI

L97 ANSWER 126 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI **Oxidative stabilities** of low-linolenate, high-stearate and common soybean oils
SO JAOCS, J. Am. Oil Chem. Soc. (1988), 65(8), 1334-8
CODEN: JJASDH
AU White, Pamela J.; Miller, Lynne A.
AN 1988:548197 HCAPLUS
DN 109:148197

L97 ANSWER 127 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI High-temperature stabilities of low-linolenate, high-stearate and common soybean oils
SO JAOCS, J. Am. Oil Chem. Soc. (1988), 65(8), 1324-7
CODEN: JJASDH

AU Miller, Lynne A.; White, Pamela J.
AN 1988:548195 HCAPLUS
DN 109:148195

L97 ANSWER 128 OF 170 MEDLINE DUPLICATE 47
TI Sequential oxidation and glutathione addition to 1,4-benzoquinone: correlation of toxicity with increased glutathione substitution.
SO MOLECULAR PHARMACOLOGY, (1988 Dec) 34 (6) 829-36.
Journal code: 0035623. ISSN: 0026-895X.
AU Lau S S; Hill B A; Highet R J; Monks T J
AN 89070548 MEDLINE

L97 ANSWER 129 OF 170 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. DUPLICATE 48
TI GENETIC ANALYSIS OF TOCOPHEROL AND FATTY ACID COMPOSITION IN SUNFLOWER SEEDS.
SO GENETIKA, (1988) 24 (3), 518-527.
CODEN: GNKAA5. ISSN: 0016-6758.
AU POPOV P S; D'YAKOV A B; BORODULINA A A; DEMURIN YA N
AN 1988:482435 BIOSIS

L97 ANSWER 130 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Subtilisin - an enzyme designed to be engineered;
alteration of catalysis, substrate specificity, pH profile, and stability
SO Trends Biochem.Sci.; (1988) 13, 8, 291-97
CODEN: TBSCDB
AU Wells J A; Estell D A
AN 1988-11019 BIOTECHDS

L97 ANSWER 131 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Genetic modification of polyunsaturated fatty acid composition in flax (Linum usitatissimum L.)
SO Proc. - World Conf. Biotechnol. Fats Oils Ind. (1988), Meeting Date 1987, 55-7. Editor(s): Applewhite, Thomas H. Publisher: AOCS, Champaign, Ill.
CODEN: 56NIAQ
AU Green, Allan G.
AN 1989:530912 HCAPLUS
DN 111:130912

L97 ANSWER 132 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New **mutant** carbonyl hydrolase enzymes;
with substitutions, deletions and/or insertions in parent protein sequence of Bacillus amyloliquefaciens subtilisin
AN 1988-03592 BIOTECHDS
PI AU 8772281 5 Nov 1987

L97 ANSWER 133 OF 170 WPIDS (C) 2003 THOMSON DERWENT
TI DNA **mutagenesis** process - by introducing restriction enzyme sites, digesting and introducing oligonucleotide(s) capable of annealing ot the restriction enzyme sites.
PI EP 247647 A 19871202 (198748)* 71p
R: AT BE CH DE FR GB IT LI LU NL SE
EP 247647 B 19910123 (199104)
R: AT BE CH DE FR GB IT LI LU NL SE
IN BOTT, R R; ESTELL, D A; FERRARI, E; HENNER, D J; WELLS, J A

L97 ANSWER 134 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Designing substrate specificity by protein engineering of electrostatic interactions;
using Bacillus amyloliquefaciens subtilisin subjected to cassette **mutagenesis**
SO Proc.Natl.Acad.Sci.U.S.A.; (1987) 84, 5, 1219-23
CODEN: PNASA6

AU Wells J A; Powers D B; Bott R R; Graycar T P; Estell D A
AN 1987-05609 BIOTECHDS

L97 ANSWER 135 OF 170 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI A ROLE OF MIXED-FUNCTION OXIDATION REACTIONS IN THE ACCUMULATION OF ALTERED ENZYME FORMS DURING AGING.
SO J. Am. Geriatr. Soc., (1987) 35 (10), 947-956.
CODEN: JAGSAF. ISSN: 0002-8614.
AU OLIVER C N; LEVINE R L; STADTMAN E R
AN 1988:98522 BIOSIS

L97 ANSWER 136 OF 170 MEDLINE DUPLICATE 49
TI Chemical modification of bovine liver rhodanese with tetrathionate: differential effects on the sulfur-free and sulfur-containing catalytic intermediates.
SO BIOCHIMICA ET BIOPHYSICA ACTA, (1987 Jan 5) 911 (1) 102-8.
Journal code: 0217513. ISSN: 0006-3002.
AU Prasad A R; Horowitz P M
AN 87076702 MEDLINE

L97 ANSWER 137 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI New human alpha-1 antitrypsin analog with altered 358 amino acid; useful as an anticoagulant and elastase inhibitor with improved **oxidation stability** in vivo; production using transformed Escherichia coli strain etc.
AN 1986-03331 BIOTECHDS
PI WO 8600337 16 Jan 1986

L97 ANSWER 138 OF 170 MEDLINE DUPLICATE 50
TI New crystalline derivatives of bovine liver rhodanese.
SO BIOCHEMISTRY INTERNATIONAL, (1986 May) 12 (5) 733-40.
Journal code: 8100311. ISSN: 0158-5231.
AU Berni R; Cannella C; Monaco H L; Rossi G L
AN 86269059 MEDLINE

L97 ANSWER 139 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Genetic conversion of linseed oil from industrial to edible quality; following genetic engineering of linseed plant; **mutagenesis** (conference abstract)
SO J.Am.Oil Chem.Soc.; (1986) 63, 4, 464
CODEN: JAOCA7
AU Green A G
AN 1986-08618 BIOTECHDS

L97 ANSWER 140 OF 170 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
TI GENETIC CONVERSION OF LINSEED OIL FROM INDUSTRIAL TO EDIBLE QUALITY.
SO DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH PLANT BREEDING SYMPOSIUM, LINCOLN, NEW ZEALAND, FEBRUARY 17-20, 1986. AGRON SOC N Z SPEC PUBL. (1986 (RECD 1987)) 0 (5), 266-270.
CODEN: SPAZD9.
AU GREEN A G
AN 1987:205784 BIOSIS

L97 ANSWER 141 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Protein engineering of industrial enzymes; e.g. subtilisin and glucose-isomerase; genetic engineering application (conference paper)
SO World Biotech Rep.; (1986) 1, B1-B8
AU Hartley B S
AN 1987-06558 BIOTECHDS

L97 ANSWER 142 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Protein engineering of subtilisn; enzyme engineering for improved biocatalysis (conference paper)

SO Genet.Eng.Tech; (1986) 3 pp
AU Fersht A R; Russell A J; Thomas P G
AN 1988-00530 BIOTECHDS

L97 ANSWER 143 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Prokaryotic carbonyl-hydrolase for use with surfactants; prepared by cultivation of host transformed with recombinant vector; Bacillus subtilisin etc. gene cloning for use in detergent
AN 1985-03667 BIOTECHDS
PI EP 130756 9 Jan 1985

L97 ANSWER 144 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Active site modified protease .alpha.1-antitrypsin inhibitors
SO Eur. Pat. Appl., 37 pp.
CODEN: EPXXDW
IN Barr, Philip J.; Hallewell, Robert A.; Rosenberg, Steven; Brake, Anthony J.
AN 1986:221366 HCAPLUS
DN 104:221366

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 164719	A2	19851218	EP 1985-107126	19850610
EP 164719	A3	19860806		
EP 164719	B1	19920506		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
US 4732973	A	19880322	US 1984-620408	19840614
US 4752576	A	19880621	US 1984-620662	19840614
AT 75753	E	19920515	AT 1985-107126	19850610

L97 ANSWER 145 OF 170 MEDLINE DUPLICATE 52
TI Engineering an enzyme by site-directed **mutagenesis** to be resistant to chemical oxidation.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1985 Jun 10) 260 (11) 6518-21.
Journal code: 2985121R. ISSN: 0021-9258.
AU Estell D A; Graycar T P; Wells J A
AN 85207651 MEDLINE

L97 ANSWER 146 OF 170 MEDLINE DUPLICATE 53
TI Isolation and properties of recombinant DNA produced variants of human alpha 1-proteinase inhibitor.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1985 Apr 10) 260 (7) 4384-9.
Journal code: 2985121R. ISSN: 0021-9258.
AU Travis J; Owen M; George P; Carrell R; Rosenberg S; Hallewell R A; Barr P J
AN 85157604 MEDLINE

L97 ANSWER 147 OF 170 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 54
TI **OXIDATIVE STABILITY** OF HIGH OLEIC SUNFLOWER AND SAFFLOWER OILS.
SO J AM OIL CHEM SOC, (1985) 62 (3), 523-525.
CODEN: JAOCA7. ISSN: 0003-021X.
AU PURDY R H
AN 1985:324110 BIOSIS

L97 ANSWER 148 OF 170 LIFESCI COPYRIGHT 2003 CSA
TI Effect of heat treatment and lipoxygenase activity on soybean oil quality. BELTSVILLE SYMPOSIUM X. BIOTECHNOLOGY FOR SOLVING AGRICULTURAL PROBLEMS, MAY 5-9, 1985. ABSTRACTS.
SO (1985) p. 14. Summary only..
Meeting Info.: 10. Beltsville Symposium on Biotechnology for Solving Agricultural Problems. Beltsville, MD (USA). 5-9 May 1985.
AU Hildebrand, F.D.; Altschuler, M.; Wang, X.; Polacco, J.L.; Ma, Y.; Collins, G.B.; Lazzeri, P.A.; Dahmer, M.; Benzion, G.; et al..

AN 85:7076 LIFESCI

L97 ANSWER 149 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Polypeptide **oxidative stable** serine protease-inhibitor;
human alpha-1-antitrypsin gene cloning and expression in Saccharomyces carlsbergensis/Saccharomyces cerevisiae hybrid
AN 1986-02268 BIOTECHDS
PI EP 164719 18 Dec 1984

L97 ANSWER 150 OF 170 MEDLINE DUPLICATE 55
TI The high resolution three-dimensional structure of bovine liver rhodanese.
SO FUNDAMENTAL AND APPLIED TOXICOLOGY, (1983 Sep-Oct) 3 (5) 370-6. Ref: 28
Journal code: 8200838. ISSN: 0272-0590.
AU Hol W G; Lijk L J; Kalk K H
AN 84058801 MEDLINE

L97 ANSWER 151 OF 170 MEDLINE DUPLICATE 56
TI Interaction of rhodanese with intermediates of oxygen reduction.
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L97 ANSWER 152 OF 170 HCAPLUS COPYRIGHT 2003 ACS
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L97 ANSWER 153 OF 170 NTIS COPYRIGHT 2003 NTIS
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PD Oct 1981
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L97 ANSWER 154 OF 170 MEDLINE
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L97 ANSWER 155 OF 170 MEDLINE
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Report No.: PIP-004326; POP-00089585.
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L97 ANSWER 156 OF 170 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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L97 ANSWER 157 OF 170 WPIDS (C) 2003 THOMSON DERWENT
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SE 7906116 A 19800218 (198010)
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L97 ANSWER 158 OF 170 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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L97 ANSWER 160 OF 170 MEDLINE DUPLICATE 57
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L97 ANSWER 161 OF 170 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 58
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L97 ANSWER 162 OF 170 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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L97 ANSWER 163 OF 170 HCAPLUS COPYRIGHT 2003 ACS
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L97 ANSWER 164 OF 170 MEDLINE DUPLICATE 59
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PI DE 1964792	A	19710701	DE 1969-1964792	19691224

L97 ANSWER 166 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Enzyme-containing, stain-removing detergents
SO Ger. Offen., 41 pp.
CODEN: GWXXBX
IN Goette, Ernst; Krings, Peter; Schwuger, Milan J.; Borggrefe, Gerhard
AN 1971:127997 HCAPLUS
DN 74:127997

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 1942236	A	19710304	DE 1969-1942236	19690820

L97 ANSWER 167 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Interaction of the 5-methylphenazinium cation radical with deoxyribonucleic acid
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CODEN: BICHAW; ISSN: 0006-2960
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AN 1969:111579 HCAPLUS
DN 70:111579

L97 ANSWER 168 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI Relation between composition and **oxidation stability** of lubricating oils
SO Ropa a Uhlie (1969), 11(6), 297-353
CODEN: ROUHAY; ISSN: 0035-8231
AU Vesely, Vaclav
AN 1970:4895 HCAPLUS
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L97 ANSWER 169 OF 170 HCAPLUS COPYRIGHT 2003 ACS
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SO Corsi e Seminari di Chimica, Consiglio Nazionale delle Ricerche e Fondazione F. Giordani (1968), (8), 99-101
CODEN: CSECB7; ISSN: 0579-0670
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L97 ANSWER 170 OF 170 HCAPLUS COPYRIGHT 2003 ACS
TI "Free" sulfur content of proteins. Response
SO Textile Research Journal (1967), 37(11), 1004
CODEN: TRJOA9; ISSN: 0040-5175
AU Gupta, Kamlesh
AN 1968:84256 HCAPLUS
DN 68:84256

=> d ab 4,8,13,14,22,27,29,30,38,39,42,44,56,64,71,78,82,85,88,99,100,102,123,160

L97 ANSWER 4 OF 170 WPIDS (C) 2003 THOMSON DERWENT
AB WO 200069901 A UPAB: 20010116
NOVELTY - A non-naturally occurring insulin activity (IA) protein (I) comprising an amino acid sequence containing substitutions of amino acid residues when compared to the amino acid sequence of a naturally occurring human insulin, which has altered properties when compared to insulin and which binds to a cell comprising an insulin receptor, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a non-naturally occurring (I) conformer (II) having a three-dimensional back bone structure that substantially corresponds to the three-dimensional back bone structure of human insulin and which has 98% identity with the amino acid sequence of human insulin;

(2) a recombinant nucleic acid (II) encoding (I);

(3) an expression vector (III) comprising (II);

(4) a host cell (IV) comprising (II) or (III);

(5) the preparation of (I); and

(6) a pharmaceutical composition comprising (I).

ACTIVITY - Antidiabetic; immunosuppressive.

No supporting data is given.

MECHANISM OF ACTION - Insulin-like protein; growth and metabolism regulator.

USE - (I) is useful for treating insulin responsive conditions and disorders of carbohydrate metabolism such as type 1 or type 2 diabetes (claimed). The nucleic acids encoding (I) are used in gene therapy techniques for treating the above mentioned conditions.

ADVANTAGE - (I) has altered biological properties compared to insulin, is more stable than insulin and has enhanced **oxidative**, alkaline and thermal **stability** than insulin.

Dwg.0/10

L97 ANSWER 8 OF 170 MEDLINE DUPLICATE 2
AB The gene of the NAD-dependent formate dehydrogenase (FDH) from the yeast Candida boidinii was cloned by PCR using genomic DNA as a template. Expression of the gene in Escherichia coli yielded functional FDH with about 20% of the soluble cell protein. To confirm the hypothesis of a thiol-coupled inactivation process, both cysteine residues in the primary structure of the enzyme have been exchanged by site-directed **mutagenesis** using a homology model based on the 3D structure of FDH from Pseudomonas sp. 101 and from related dehydrogenases. Compared to the wt enzyme, most of the **mutants** were significantly more **stable** towards **oxidative** stress in the presence of Cu(II) ions, whereas the temperature optima and kinetic constants of the enzymatic reaction are not significantly altered by the **mutations**. Determination of the Tm values revealed that the stability at temperatures above 50 degrees C is optimal for the native and the recombinant wt enzyme (Tm 57 degrees C), whereas the Tm values of the **mutant** enzymes vary in the range 44-52 degrees C. Best results in initial tests concerning the application of the enzyme for regeneration of NADH in biotransformation of trimethyl pyruvate to Ltert leucine were obtained with two **mutants**, FDHC23S and FDHC23S/C262A, which are significantly more stable than the wt enzyme.

L97 ANSWER 13 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB The enzyme engineering of bacterial alpha-amylases (EC-3.2.1.1) is reviewed with respect to the following topics: (1) industrial uses, such as in surfactants and for starch liquefaction; (2) classification of enzymes displaying alpha-amylase activity; (3) structure, especially relating to (a) calcium and sodium ions, (b) chloride ions, and (c) the active site cleft; (4) sequence, including conserved regions; (5) catalytic mechanism; (6) stability engineering, including (a) the effect of metal ions and metal ion chelators, (b) the development of hybrid

enzymes, (c) stabilization by the introduction of proline residues, (d) role of residues 133 and 209 in thermostability of Bacillus sp. alpha-amylase, (e) random **mutagenesis**, (f) calcium sites, (g) deamidation, (h) **oxidation stability**, and (i) clustering of stabilizing **mutations**; and (7) engineering pH-activity profiles. It is predicted that the focus of research will switch from stability engineering to the development of enzymes with specific pH-activity profiles and tailor-made substrate and product specificities. (91 ref)

L97 ANSWER 14 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB Lipases (EC-3.1.1.3) have many industrial uses, e.g. surfactants, oils and fats, baking, organic synthesis, hard surface cleaning, and the leather and paper industries. The following topics of lipase enzyme engineering are reviewed: variants addressing the active site; variants addressing activity (including specific activity, substrate type specificity, chain length specificity, and positional specificity and enantioselectivity); variants addressing stability, including thermostability, protease **stability** and **oxidative stability**; variants addressing lid function; variants addressing macroscopic substrate interaction; variants addressing calcium binding; variants addressing use in surfactants; variants addressing X-ray structure and dynamics; and **mutation** strategy. Also provided are tables listing selected lipase X-ray structures and protein engineering topics, a sequence alignment of Rhizomucor miehei, Rhizopus oryzae, Penicillium camembertii and Humicola lanuginosa lipases, a general reaction scheme for lipase-substrate interaction, and diagrams of lipase overall structure, lipid contact zone, ribbon structure, active site confirmation and acyl binding cleft. (122 ref)

L97 ANSWER 22 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB A DNA sequence (I) encoding a maleate-isomerase (II) (EC-5.2.1.1) optionally expressed recombinantly in Escherichia coli is claimed. The enzyme exhibits a good resistance to oxidation - its activity is not inhibited by more than 40% when a cell free extract from recombinant E. coli expressing (II) is incubated in the presence of 30 mM hydrogen peroxide at 30 deg for 1 hr. Also new are: vectors and host cells harboring (I); a method for the recombinant production of (II); and a method for the preparation of fumaric acid which involves adding a roughly purified (II) to an aq. solution of malic acid, and allowing isomerization to take place. In an example, site-specific **mutagenesis** was achieved on the maleate-isomerase gene of Serratia marcescens IFO3736 using recombinant polymerase chain reaction. The amplified fragments were cloned into vector plasmid pKK223-3 and expressed in E. coli JM109 to give E. coli ECSMW and ECMC1. (12pp)

L97 ANSWER 27 OF 170 MEDLINE DUPLICATE 10
AB Engineering enzyme activity has been challenging because of uncertainties in structure-function relationships and difficulties in screening a large number of **mutant** enzymes. A product capture strategy using phage display is presented here for the selection of improved enzymes from a large library of variants (>10(9) independently derived **mutants**). Subtiligase, a double **mutant** of subtilisin BPN' that catalyzes the ligation of peptides, was displayed on phage. Twenty-five active site residues were randomly **mutated** in groups of four or five to yield six different libraries that were independently sorted. Variants that ligated a biotin peptide onto their own extended N termini were selectively captured. **Mutant** subtiligases were identified that had increased ligase activity. The selection also yielded unexpected subtiligase **mutants** having residues known to improve the **stability** and **oxidative** resistance of wild-type subtilisin. These studies are exemplary for the use of phage to improve enzyme function when it is closely linked to a selectable catalytic event.

L97 ANSWER 29 OF 170 MEDLINE DUPLICATE 11
AB The Coprinus cinereus (CiP) heme peroxidase was subjected to multiple rounds of directed evolution in an effort to produce a **mutant** suitable for use as a dye-transfer inhibitor in laundry detergent. The wild-type peroxidase is rapidly inactivated under laundry conditions due to the high pH (10.5), high temperature (50 degrees C), and high peroxide concentration (5-10 mM). Peroxidase **mutants** were initially generated using two parallel approaches: site-directed **mutagenesis** based on structure-function considerations, and error-prone PCR to create random **mutations**. **Mutants** were expressed in Saccharomyces cerevisiae and screened for improved stability by measuring residual activity after incubation under conditions mimicking those in a washing machine. Manually combining **mutations** from the site-directed and random approaches led to a **mutant** with 110 times the thermal stability and 2.8 times the **oxidative stability** of wild-type CiP. In the final two rounds, **mutants** were randomly recombined by using the efficient yeast homologous recombination system to shuffle point **mutations** among a large number of parents. This in vivo shuffling led to the most dramatic improvements in **oxidative stability**, yielding a **mutant** with 174 times the thermal stability and 100 times the **oxidative stability** of wild-type CiP.

L97 ANSWER 30 OF 170 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AB Chemical modification and **mutagenesis** of methionines in recombinant human granulocyte colony-stimulating factor (G-CSF) were investigated. Selective oxidation of G-CSF by H2O2 and t-butyl hydroperoxide leads to generation of different oxidized forms. Four modified forms were isolated and shown to contain 1 to 4 oxidized methionyl residues. All methionines in G-CSF are reactive, with reaction kinetics following the order of Met1>Met138>Met127mchgt>Met122. H2O2 oxidation of Met122 is relatively slow and is biphasic with a faster second reaction phase being affected by the oxidation of Met127. All oxidized forms retain gross G-CSF conformation similar to that of the native molecule and are able to bind the soluble G-CSF receptor. However, G-CSF form oxidized at both Met127 and Met122 is unstable and exhibits decreased ability to dimerize the receptor after exposure to acid or elevated temperature. All modified forms, except Met1-oxidized G-CSF, also show significantly lower biological activity. Our data suggest that Met138 is solvent accessible and its surrounding microenvironment may be critical for G-CSF function, whereas Met127 is less accessible to solvent and Met122 is near the hydrophobic core. Oxidation at both Met127 and Met122 results in alterations of G-CSF structure that affect the apparent molecular size, polarity, and stability and lead to the loss of G-CSF biological function. G-CSF variants with Leu replacement at Met127 or at Met138 are not completely resistant to oxidation-induced inactivation, while the variant with Leu replacement at both sites is more stable and can retain in vitro biological activity following oxidation.

L97 ANSWER 38 OF 170 HCAPLUS COPYRIGHT 2003 ACS
AB A review with 66 refs. Two reasons mitochondrial cytochrome c is so popular a research subject is its structural stability and its versatility as a model for studies in disciplines as varied as protein chem. and inorg. chem. Our own interest in cytochrome c has centered mainly on its structure-function and structure-stability relationships. This line of research was strongly reinforced when it became possible to alter the primary structure of the protein through site-directed **mutagenesis** and to test in the artificial **mutants** various explanations that had been postulated to account for the chem. behavior of cytochrome c. In this article, we discuss the problems, hypotheses and expts. designed to investigate the structural role of the heme iron and the effects of its **oxidn**. state on the **stabilities** of the iron-protein bonds and the whole cytochrome c mol.

L97 ANSWER 39 OF 170 HCAPLUS COPYRIGHT 2003 ACS
AB The gene of the NAD-dependent formate dehydrogenase from the yeast Candida boidinii was cloned by polymerase chain reaction and expressed in E. coli. The recombinant enzyme was stabilized by site-directed **mutagenesis** . Two cysteine residues probably located at the surface of the protein were exchanged against various aliph. amino acids and the **mutant** enzymes were tested for **stability** against **oxidn**. by air in the presence of catalytic amts. of Cu(II). All **mutants** were significantly more **stable** against **oxidative** stress than the wild-type enzyme. Addnl., the **mutant** enzymes were characterized with respect to their temp. optima, kinetic consts. and activation energy of the enzymic reaction. The results of these studies indicated that the catalytic properties of the **mutant** enzymes have not been altered by the **mutagenesis** compared to the wild-type enzyme.

L97 ANSWER 42 OF 170 MEDLINE DUPLICATE 16
AB Three variants of the Candida antarctica B lipase have been constructed and characterized. The variant containing the T103G **mutation**, which introduces the consensus sequence G-X-S-X-G found in most other known lipases, shows an increased thermostability but retains only half the specific activity of the native enzyme. Also in ester synthesis the activity is lowered but the specificity and enantioselectivity remains unchanged. The W104H **mutant**, in which more space is introduced into the active site, has more dramatically changed properties. Both the thermostability and the specific activity are slightly reduced but the activity and specificity in ester synthesis is highly different from the native enzyme. In general, the activity is very low and the enantioselectivity is, furthermore, highly reduced. Finally, the **mutation** M72L was introduced to increase the **oxidation** **stability** of the enzyme. This variant did exhibit an increased resistance towards oxidation but the thermostability was, unfortunately, also reduced.

L97 ANSWER 44 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB A new Termamyl-like alpha-amylase (EC-3.2.1.1) variant has an alteration in at least one property selected from: substrate specificity; binding or cleavage pattern; thermal stability; pH activity or profile; **stability** toward **oxidation**; Ca2+ dependency; or specific activity. Also claimed are: variant **mutations** in a known Bacillus licheniformis alpha-amylase; recombinant expression vectors; and transformed cells (e.g. Bacillus subtilis Bacillus licheniformis etc.) containing the new DNA. Preferred variants exhibit increased stability at low pH and low Ca2+, and have specified **mutations**. The parent Termamyl-like alpha-amylase has a specified sequence e.g. from Bacillus amyloliquefaciens, Bacillus stearothermophilus, or Bacillus spp. NCIB 12512, NCIB 12513 or 707, and may be a hybrid of two or more alpha-amylases. The following uses for the new variant are claimed: manual and automatic dishwashing, especially as a surfactant additive with other enzymes, e.g. protease, lipase, peroxidase, cellulase or other amylolytic enzymes; industrial starch processing e.g. liquefaction; sweetener production; and textile desizing. (101pp)

L97 ANSWER 56 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 19
AB E156S and V165I **mutation** were introduced into subtilisin E gene by site-directed **mutagenesis**. The **mutated** gene fragments were recombined with pBE-2 which is a shuttle vector between E. coli and Bacillus subtilis. The recombinant plasmids were used to transform B. subtilis DB104, a **mutant** strain deficient in alkaline and neutral protease, then they were expressed. They were (M222A, E156S) and (M222A, E156S, V165I). The property analysis of these enzymes revealed that the Subtilisin E E156S substitution enhenced the hydrolysis K-cat/K-m by 90 % while keeping thermal **stability** and

oxidation-resistance unchanged, however the V165I **mutation** reduced the K-cat/K-m value.

L97 ANSWER 64 OF 170 MEDLINE DUPLICATE 22
AB Thioredoxin (Trx) is a widely distributed redox protein that regulates several intracellular redox-dependent processes and stimulates the proliferation of both normal and tumor cells. We have found that when stored in the absence of reducing agents, human recombinant Trx undergoes spontaneous oxidation, losing its ability to stimulate cell growth, but is still a substrate for NADPH-dependent reduction by human thioredoxin reductase. There is a slower spontaneous conversion of Trx to a homodimer that is not a substrate for reduction by thioredoxin reductase and that does not stimulate cell proliferation. Both conversions can be induced by chemical oxidants and are reversible by treatment with the thiol reducing agent dithiothreitol. SDS-PAGE suggests that Trx undergoes oxidation to monomeric form(s) preceding dimer formation. We have recently shown by X-ray crystallography that Trx forms a dimer that is stabilized by an intermolecular Cys73-Cys73 disulfide bond. A Cys73-->Ser **mutant** Trx (C73S) was prepared to determine the role of Cys73 in **oxidative stability** and growth stimulation. C73S was as effective as Trx in stimulating cell growth and was a comparable substrate for thioredoxin reductase. C73S did not show spontaneous or oxidant-induced loss of activity and did not form a dimer. The results suggest that Trx can exist in monomeric forms, some of which are mediated by Cys73 that do not stimulate cell proliferation but can be reduced by thioredoxin reductase. Cys73 is also involved in formation of an enzymatically inactive homodimer, which occurs on long term storage or by chemical oxidation. Thus, although clearly involved in protein inactivation, Cys73 is not necessary for the growth stimulating activity of Trx.

L97 ANSWER 71 OF 170 MEDLINE DUPLICATE 26

L97 ANSWER 78 OF 170 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 29
AB Oxidation is one of the major chemical degradation pathways for protein pharmaceuticals. **Methionine**, **cysteine**, histidine, tryptophan, and tyrosine are the amino acid residues most susceptible to oxidation due to their high reactivity with various reactive oxygen species. Oxidation during protein processing and storage can be induced by contaminating oxidants, catalyzed by the presence of transition metal ions and induced by light. Oxidative modification depends on the structural features of the proteins as well as the particular oxidation mechanisms inherent in various oxidative species, and may also be influenced by pH, temperature, and buffer composition. Protein oxidation may result in loss of biological activity and other undesirable pharmaceutical consequences. Strategies to **stabilize** proteins against **oxidation** can be classified into intrinsic methods (site-directed **mutagenesis** and chemical modification), physical methods (solid vs. liquid formulations), and use of chemical additives. The optimum choice of chemical additives needs to be evaluated on the basis of the specific oxidation mechanism. Oxidation induced by the presence of oxidants in the system is referred to as a non-site-specific mechanism. Under such conditions, oxidation can be effectively inhibited by the appropriate addition of antioxidants or free radical scavengers. Metal-catalyzed oxidation is a site-specific process, in which the addition of antioxidants may accelerate the oxidation reaction. Careful screening of chelating agents has been shown to be an alternative method for preventing metal-catalyzed oxidation. (C) 1995 John Wiley & Sons, Inc.

L97 ANSWER 82 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB The following are claimed: (1) a modified lipase (I, EC-3.1.1.3) in which at least the Met at position corresponding to position 21 of wild-type Pseudomonas pseudoalcaligenes lipase (II) is replaced by another amino acid; (2) recombinant DNA encoding (I); (3) vectors containing such DNA;

and (4) hosts transformed with such DNA or vectors. Plasmid pTMPv18A contains DNA encoding (II). Single-stranded DNA is prepared and amplified by 25 cycles of the polymerase chain reaction. The amplified fragment is then digested and cloned. Vectors containing the **mutated** gene are introduced into lipid negative P. pseudoalcaligenes Ps600 so as to incorporate the gene into the genome for increased lipase production. Preferably, the wild-type lipase is from a Pseudomonas groups (I) species, it especially has at least 70% homology with (II) and has Met replaced by Leu, Ser or Ala. Compared with wild-type enzyme, (I) has improved wash performance, altered specific pH or substrate activities, and/or better **oxidation stability**. (I) are useful in surfactant compositions. (34pp)

L97 ANSWER 85 OF 170 WPIDS (C) 2003 THOMSON DERWENT
AB WO 9404697 A UPAB: 19950904
Elafin derivatives of formula (I) are new: ALA-GLN-GLU-PRO-VAL-LYS-GLY-PRO-VAL-SER- THR-LYS-PRO-GLY-SER-CYS-PRO-ILE-ILE-LEI -ILE-ARG-CYS-ALA-XXX-LEU-ASN-PRO-PRO-ASN- -ARG-CYS-LEU-LYS-ASP-THR-ASP-CYS-PRO-GLY- -ILE-LYS-LYS-CYS-CYS-GLU-GLY-SER-CYS-GLY- -MET-ALA-CYS-PHE-VAL-PRO-GLN (I) where XXX is LEU, ILE or VAL.

(I) have improved **oxidation stability** over natural elafin and thus retain their activity better under oxidative conditions.

DNA coding for elafin is **mutated** inserted into a suitable vector and then used to transform E.Coli, yeast, Bacillus subtilis or animal cells. The modified elafin is expressed when the transferred cells are cultured.

USE/ADVANTAGE - (I) are drugs with elastase inhibitors activity.
Dwg.0/4
Dwg.0/4

L97 ANSWER 88 OF 170 MEDLINE DUPLICATE 33
AB **Mutants** of rhodanese (EC 2.8.1.1) which substitute serine residues for each of the 4 cysteine residues have been studied to determine the roles of cysteines in the structure and function of the enzyme. The proteins compared in these studies were: the wild-type, C63S, C247S, C254S, C263S, C254S/C263S, and C63S/C254S/C263S. These current studies show that cysteine 247 is the only cysteine required for the activity of the enzyme. Although the other sulfhydryl groups do not participate in sulfur transfer, **mutations** of the noncatalytic cysteines result in the destabilization of the native structure of the enzyme. All the active proteins had similar kinetic parameters. **Mutants** substituting cysteine 254, compared with the other species, were: (a) more resistant than wild-type to inactivation by dithiothreitol, (b) more readily reactivated following oxidative inactivation, and (c) found to adopt conformations that show increased exposure of hydrophobic surfaces following removal of the transferable sulfur. On the other hand, cysteine to serine substitutions had very little effect on: (a) the rates of oxidative inactivation, (b) the increased fluorescence following the removal of transferable sulfur, or (c) the effectiveness of spontaneous refolding after urea denaturation. Forms of rhodanese that were formerly considered to be irreversibly oxidized can be reactivated if the protein is denatured in urea before reductants are used. It is proposed that these forms differ from reversibly oxidized states due to the inaccessibility of intramolecular disulfides to reductants and not to the formation of higher oxidation states of the protein.

L97 ANSWER 99 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB Stable enzymes can generally be obtained in several ways: screening for stability using e.g. thermophiles; chemical modification; enzyme engineering; immobilization; or the use of stabilizing additives. For industrial enzymes, the use of additives and protein engineering have found wide application. The storage stability of the surfactant

protease, Maxacal, in bleach containing high-duty powder detergents was improved by **mutating** a methionine in the active site (position 216). A few **oxidation-stable mutants** revealed the same specific wash performance as the wild-type enzyme. The operational-half-life of immobilized glucose-isomerase (EC-5.3.1.5, Maxazyme GI-immob) was increased 2- to 3-fold by **mutating** a lysine in the subunit interface (position 253) into an arginine residue. The **mutation** prevented the enzyme from dissociation after glycation of the lysine by glucose. The specific activity of the **mutant** was unaltered. Random **mutagenesis** yielded thermostable **mutants** of Bacillus licheniformis alpha-amylase (Maxamyl, EC-3.2.1.1), used in starch liquefactions, which also showed unaltered specific activity. (12 ref)

L97 ANSWER 100 OF 170 MEDLINE DUPLICATE 39
AB Microbial proteases are used extensively in a large number of industrial processes and most importantly in detergent formulations facilitating the removal of proteinaceous stains. Site-directed **mutagenesis** has been employed in the construction of subtilisin variants with improved storage and **oxidation stabilities**. It is shown that in spite of significant structural homology between subtilisins subjected to protein engineering the effects of specific **mutations** can be quite different. **Mutations** that stabilize one subtilisin may destabilize another.

L97 ANSWER 102 OF 170 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AB Protein engineering and design are reviewed as follows: (1) the development and scientific basis of protein engineering research projects; (2) the 'protein design cycle', i.e. the steps involved in protein engineering, described using a typical enzyme, e.g. a protease, as a model - (a) screening, purification and characterization, (b) cloning, expression and genetic engineering of the wild-type enzyme, (c) crystallization and structure elucidation, (d) computer modeling, (e) protein structure modifications, and (f) site-directed **mutagenesis** and evaluation of the wild-type protein; and (3) examples of protein engineering and design. Tables are provided that list: (1) protein variants produced for protein engineering projects and their abundance; (2) targets for protein engineering and design (thermostability, **stability** to **oxidation**, **stability** to heavy metals, pH stability and improved enzymatic properties); and (3) examples of engineered peptides, proteins, enzymes, artificial proteins and inhibitors, the engineering targets, and methods and results. (144 ref)

L97 ANSWER 123 OF 170 HCAPLUS COPYRIGHT 2003 ACS
AB Sulfur is unique among the main elements of living cells in that it is covalently bound to biopolymers but does not occur in the biopolymer backbone. Indeed, most of the bacterial sulfur content resides in the **methionine** and **cysteine** side-chains of proteins. The growth yield of an organism under conditions of sulfur limitation could therefore be greatly enhanced by **mutations** that substitute Met and Cys in the organism's **proteins** for **sulfur-free** amino acids. Because the saving in sulfur would increase with such accumulating **mutations**, Met and Cys changes could be progressively selected. Abundant proteins should be the prime targets of such a selection. Sulfate permease, which is abundantly produced by sulfur-starved Salmonella typhimurium, lacks Met and Cys residues also, two species of marine purple bacteria synthesize more protein than can be expected from a limited sulfate supply. This report shows that the cyanobacterium Calothrix sp. PCC 7601 encodes sulfur-depleted versions of its most abundant proteins, phycocyanin and its auxiliary polypeptides, which it specifically expresses under conditions of sulfur limitation. Although these proteins do not take part in the fixation of sulfur, their elevated synthesis affects the sulfur budget of cyanobacterial cells.

Directed evidence is thus provided that the structure of macromols. can be subject to metabolic optimization.

L97 ANSWER 160 OF 170 MEDLINE DUPLICATE 57

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         22918 PRY<=2000
                 (PRY<=2000)
         48177 PY=>2001
                 (PY=>2001)
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               PY=>2001

FILE 'HCAPLUS'
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        370231 PRY<=2000
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               PY=>2001

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PROCESSING COMPLETED FOR L101
L102         16 DUP REM L101 (3 DUPLICATES REMOVED)

=> d tot

L102  ANSWER 1 OF 16  BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI    Computer-implemented prediction of an amino acid sequence compatible with
      a specified three-dimensional structure of a protein or peptide involves
      at least one scoring function calculating step;
         bioinformatics and proteomics with application in drug design and gene
         therapy
AU    BECKER O M; TOPF M
```

AN 2002-12912 BIOTECHDS
PI **WO** 2002014875 **21 Feb 2002**

L102 ANSWER 2 OF 16 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Detergent composition containing phenol-oxidizing enzyme, useful for stain removal, derived from a precursor enzyme of Stachybotrys; recombinant enzyme production, useful in surfactant, paper and pulp bleaching, cosmetic, food, feedstuff, starch and alcohol production, deodorization, sanitation, waste-water treatment, enzyme electrode, polymer and adhesive
AU AEHLE W; CONVENTS D; DOORNINK M; VAN GASTEL F; RODRIGUEZ A M; TOPPOZADA A; DE VRIES C H; WANG H
AN 2002-12600 BIOTECHDS
PI **WO** 2002020711 **14 Mar 2002**

L102 ANSWER 3 OF 16 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
TI Novel endoglucanase III (EGIII)-like cellulase variant comprising substitution/deletion at positions corresponding to specific residues in EGIII from Trichoderma reesei, useful for treating cellulose containing textile; vector-mediated gene transfer and expression in host cell, surfactant, cellulose, site-directed **mutagenesis**, polymerase chain reaction and DNA primer for use in textile industry
AU MITCHINSON C; ROPP T H; SWANSON B A
AN 2002-11535 BIOTECHDS
PI **WO** 2002012464 **14 Feb 2002**

L102 ANSWER 4 OF 16 HCAPLUS COPYRIGHT 2003 ACS
TI Bacillus strains KSM-36 and KSM-38 .alpha.-amylase variants with altered properties
SO PCT Int. Appl., 69 pp.
CODEN: PIXXD2
IN Andersen, Carsten
AN 2002:293820 HCAPLUS
DN 136:306025

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002031124	A2	20020418	WO 2001-DK668	20011012 <--
WO 2002031124	A3	20030227		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

AU 2002010380	A5	20020422	AU 2002-10380	20011012 <--
EP 1326965	A2	20030716	EP 2001-978207	20011012 <--

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

L102 ANSWER 5 OF 16 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
TI Tertiary structure modeling of Bacillus .alpha.-amylases and construction of variants with altered solubility and related enzymic properties
SO PCT Int. Appl., 152 pp.
CODEN: PIXXD2
IN Andersen, Carsten; Borchert, Torben Vedel; Nielsen, Bjarne Ronfeldt
AN 2001:676914 HCAPLUS
DN 135:238614

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001066712	A2	20010913	WO 2001-DK144	20010307 <--

WO 2001066712 A3 20020418
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
EP 1263942 A2 20021211 EP 2001-911458 20010307 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
US 2003129718 A1 20030710 US 2001-925576 20010809 <--

L102 ANSWER 6 OF 16 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
TI Method for obtaining proteins having improved stability characteristics
SO PCT Int. Appl., 42 pp.
CODEN: PIXXD2
IN Day, Anthony G.; Mitchinson, Colin; Shaw, Andrew
AN 2001:489425 HCAPLUS
DN 135:103326

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
WO 2001047956	A2	20010705	WO 2000-US33878	20001214	<--
WO 2001047956	A3	20020214			

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1240524	A2	20020918	EP 2000-984363	20001214	<--

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

L102 ANSWER 7 OF 16 HCAPLUS COPYRIGHT 2003 ACS
TI Dip-molded medical devices from cis-1,4-polyisoprene
SO PCT Int. Appl., 21 pp.
CODEN: PIXXD2
IN Mcglothlin, Mark W.; Schmid, Eric V.
AN 2001:730512 HCAPLUS
DN 135:278072

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
WO 2001072158	A1	20011004	WO 2000-US8167	20000327	<--

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EP 1296576	A1	20030402	EP 2000-921478	20000327	<--

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL

L102 ANSWER 8 OF 16 HCAPLUS COPYRIGHT 2003 ACS
TI Stabilization of proteins and enzymes by tyrosyl-tyrosyl crosslinking

SO PCT Int. Appl., 140 pp.
CODEN: PIXXD2
IN Marshall, Christopher P.; Hoffman, Alexander; Errico, Joseph P.; Marshall, Paul B.
AN 2001:300896 HCAPLUS
DN 134:323140

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001029247	A1	20010426	WO 2000-US28595	20001016 <--
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1282722	A1	20030212	EP 2000-973574	20001016 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL				
US 2002061549	A1	20020523	US 2001-837235	20010418 <--

L102 ANSWER 9 OF 16 HCAPLUS COPYRIGHT 2003 ACS
TI Genetic engineering of **sulfur free enzymes** with wild type activity and oxidation resistance
SO PCT Int. Appl., 49 pp.
CODEN: PIXXD2
IN Iwakura, Masahiro
AN 2001:12594 HCAPLUS
DN 134:67171

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001000797	A1	20010104	WO 2000-JP2112	20000331 <--
W: JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1199355	A1	20020424	EP 2000-913052	20000331 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI, CY				

L102 ANSWER 10 OF 16 WPIDS (C) 2003 THOMSON DERWENT
TI Screening for protein genes secreted by microorganism, in particular bacteria and fungi involves using immunoassay techniques to identify clones expressing genes encoding proteins secreted from the organism.

PI	WO 2001098484 A1	20011227 (200216)* EN	43p C12N015-10	<--
	RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW			
	W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW			
	AU 2001065836 A	20020102 (200230)	C12N015-10	<--
	US 2002106651 A1	20020808 (200254)	C12Q001-68	<--
	EP 1297123 A1	20030402 (200325) EN	C12N015-10	<--
	R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR			

IN JORGENSEN, B R; NIELSEN, P

L102 ANSWER 11 OF 16 WPIDS (C) 2003 THOMSON DERWENT
TI Generation of recombinant nucleic acid molecules for screening for novel polypeptides e.g. therapeutic peptides, comprises using fragments with non-extendable 3' ends as templates only, rather than as templates and primers.

PI WO 2001085929 A2 20011115 (200205)* EN 34p C12N015-10 <--
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2001059490 A 20011120 (200219) C12N015-10 <--
EP 1280894 A2 20030205 (200310) EN C12N015-10 <--
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
US 6534292 B1 20030318 (200322) C12P019-34 <--
IN VOLKOV, A

L102 ANSWER 12 OF 16 WPIDS (C) 2003 THOMSON DERWENT
TI New laccase variants from Coprinus and Myceliophthora thermophila with improved **oxidative stability**, useful for paper strengthening, dye transfer inhibition, bleaching of textiles and waste water treatment.
PI WO 2001083761 A1 20011108 (200205)* EN 154p C12N015-53 <--
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001054622 A 20011112 (200222) C12N015-53 <--
IN DANIELSEN, S; SCHNEIDER, P; SVENDSEN, A

L102 ANSWER 13 OF 16 WPIDS (C) 2003 THOMSON DERWENT
TI Electrical oil comprises blend of nitrogen and **sulfur-free** paraffinic or naphthenic base oil and hydrofined light gas oil.
PI WO 2001054138 A1 20010726 (200149)* EN 15p H01B003-20 <--
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE TR
W: CA JP NO
US 6355850 B1 20020312 (200221) H01B003-22 <--
NO 2002003430 A 20020910 (200275) H01B000-00 <--
EP 1264317 A1 20021211 (200301) EN H01B003-20 <--
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR
IN ANGELO, J B; BAYS, T L

L102 ANSWER 14 OF 16 WPIDS (C) 2003 THOMSON DERWENT
TI Method of making and identifying low erucic acid **mutant** meadowfoam plant comprising contacting meadowfoam seeds with **mutagen**, harvesting second-generation seeds from first-generation plants and measuring erucic acid content of seeds.
PI WO 2001024617 A1 20010412 (200137)* EN 18p A01H005-00 <--
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000069450 A 20010510 (200143) A01H005-00 <--
IN CRANE, J M; KNAPP, S J

L102 ANSWER 15 OF 16 WPIDS (C) 2003 THOMSON DERWENT
TI Stereostructure of decarbamylase obtainable by X-ray crystal structure analysis for design of variants with stability to heat and air oxidation, optimal pH for enzyme reaction, improved specific activity, for use e.g. in organic chemistry.
PI WO 2001016337 A1 20010308 (200125)* JA 53p C12N015-60 <--
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE

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        W: CN IN KR SG US
     JP 2001069981 A  20010321 (200132)       25p    C12N015-09      <--
     EP 1209234    A1 20020529 (200243)  EN          C12N015-60      <--
        R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
     KR 2002037044 A  20020517 (200273)             C12N009-78      <--
     CN 1376202    A  20021023 (200313)             C12N015-60      <--
IN   IKENAKA, Y; ISHII, K; MORIKAWA, S; NAKAI, T; NANBA, H; TAKAHASHI, S;
     YAJIMA, K

L102 ANSWER 16 OF 16  WPIDS (C) 2003 THOMSON DERWENT
TI   Microorganism secreting out lipid comprising  unsaturated fatty acids in
     balls, for use in drugs, functional foods, cosmetics and feeds.
PI   WO 2001012780 A1 20010222 (200123)* JA   41p    C12N001-14      <--
        RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
        W: AU CA JP KR US
     AU 2000064761 A  20010313 (200134)             C12N001-14      <--
     EP 1122304    A1 20010808 (200146)  EN          C12N001-14      <--
        R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
     KR 2001073210 A  20010731 (200209)             C12N001-14      <--
     JP 2001517665 X  20030311 (200319)             C12N001-14      <--
IN   AKIMOTO, K; KAWASHIMA, H; SHIMIZU, S


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